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How important are big trees for biodiversity: a call to the local community of Lousada

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Mestrado em
Ecologia e Gestão Ambiental

Dissertação orientada por:
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2019

"Keep doing the right thing for the planet, yes, but also keep trying to save what you love specifically—a community, an institution, a wild place, a species that's in trouble—and take heart in your small successes. Any good thing you do now is arguably a hedge against the hotter future, but the really meaningful thing is that it's good today. *As long as you have something to love, you have something to hope for."*

Jonathan Franzen in "*The End of the end of the Earth*", 2018

AGRADECIMENTOS

Este projeto, e consequentemente, esta dissertação, é fruto de um caminho que tenho vindo a traçar, desde 2014, ligado à preservação de madeira morta (em todas as suas vertentes) e da sua biodiversidade associada. Trago comigo não só conhecimentos, mas também amigos e colegas com quem os tenho partilhado.

À Cristina Branquinho e especialmente à Ana Luz, orientadora e coorientadora desta dissertação, muito obrigado por aceitarem este desafio de não só me orientarem numa área de investigação um pouco diferente do que estão acostumadas, mas também por me desafiarem diariamente a alargar os horizontes pessoais e científicos, permitindo-me crescer de uma forma inegável durante o processo de execução desta dissertação, o qual estou eternamente grato.

Ao Sr. Vereador do Ambiente do Município de Lousada. Dr. Manuel Nunes, obrigado por ser uma fonte de inspiração neste mundo, lutando diariamente por um mundo melhor promovendo medidas únicas e com grandes resultados. Obrigado ainda pela confiança e as oportunidades que me tem facultado desde 2017 na criação e adoção de novas abordagens ligadas à preservação do património natural, integrando-me numa equipa multidisciplinar única com quem tenho todo o prazer de trabalhar.

A esta equipa, o Setor de Conservação da Natureza e de Educação Ambiental de Lousada, ao André Couto, ao Pedro Sá, ao Rafael Marques, ao Diego Alves, à Daniela Barbosa, ao Luís Cunha e ao Ernesto Gonçalves, agradeço todo o apoio diário em Lousada e a amizade necessária para alcançarmos, em conjunto, tudo o que temos conseguido.

Obrigado a todos os voluntários que me acompanharam no campo, à Mathilde Claes, ao Gonçalo Abreu, à Marta Pinto, à Eduarda Barbosa, à Cláudia Silva, ao João Pereira, à Mariana Cardoso, ao Pedro Freitas, ao Ivo Rosa, à Cláudia Mendes, à Mariana Carqueijeiro, à Inês Ferreira, ao Francisco Leal, à Joana Rocha e à Joana Pereira, por ajudarem não só na recolha de dados mas que o esforço todo valesse a pena.

Obrigado aos Lousadenses que sempre abriram as portas de suas casas para partilhar histórias e preocupações ambientais (enquanto se media uma ou outra árvore pelo caminho). A disponibilidade e interesse de cada uma destas pessoas é o que permite realmente, no futuro, alcançar mudanças sustentáveis para o nosso planeta.

To Jonas Hagge, Jörg Müller, Arno Thomaes, Alessandro Campannaro, Marcos Mendéz, Maria Fremlin and all the international colleagues that work in deadwood conservation and saproxylic organisms and that, since 2014, have helped and hosted me within their organisations and projects, allowing me to grow beyond everything that I've ever dreamed of: Thank you for all the help but above all, thank you for showing me this amazing world, that it is possible to be innovative in science and that its use is a vital piece in a changing world.

Aos meus colegas do Projeto VACALOURA.pt, à Tatiana Moreira, ao Gonçalo Abreu, ao João Carvalho, à Carla Rego, à Helena Ceia, ao Carlos Fonseca e à Ana Rita Gonçalves, obrigado por aceitarem começar um projeto voluntário pioneiro no nosso país e que me tem feito tão orgulhoso de nós próprios, motivando tantos outros a mudar o seu pequeno mundo em prol de um conjunto de espécies tão singulares.

À BioLiving, minha casa, e aos meus colegas e amigos que nela também encontram um porto de abrigo, obrigado por toda a motivação diária e determinação para conseguirmos mudar o mundo como temos feito.

À minha irmã, Ana, o meu pilar neste caminho, obrigado por estares sempre presente nos bons e nos maus momentos. À minha família, o chão que segura o meu mundo, obrigado por me permitirem e motivarem a seguir os meus sonhos desde que me conheço, sendo a pessoa feliz que sou. Aos meus amigos, minha família emprestada, obrigado por me motivarem diariamente a alcançar os meus objetivos pessoais e por ter o prazer de vos ter comigo.

Por fim, obrigado Milene Matos. Obrigado por toda a orientação, integração, ajuda, incentivo e amizade durante os últimos 5 anos. Obrigado por fazeres parte de cada um destes conjuntos de pessoas que referi anteriormente e por tudo o que aprendi contigo. Obrigado pela pessoa que sou e por tudo o que tenho alcançado.

RESUMO

O ser humano tem influências diretas sobre a restante biodiversidade que coexiste no Planeta Terra. As suas ações têm levado a alterações sem precedentes, principalmente como resultado de destruição ou degradação de ecossistemas que consequentemente levam a perdas de biodiversidade. Árvores de grande porte (AGP) (neste estudo, consideradas aquelas com Perímetro à Altura do Peito (PAP) superior a 150 cm) estão ligadas ao ser humano do ponto de vista social, económico mas também ecológico. Estas árvores desempenham funções ecológicas essenciais nos regimes hidrológicos e micro/mesoclimáticos, no armazenamento de carbono e nos ciclos de nutrientes, proporcionando também habitat para uma multiplicidade de seres vivos. AGP suportam estruturas como cavidades e ramos mortos, geralmente chamados de microhabitats que ocorrem em árvores (MAs) que são locais para alimentação, esconderijo e nidificação de milhares de espécies. As árvores que suportam MAs são elementos-chave à escala da paisagem, já que podem abrigar muitas espécies especializadas de flora e fauna ameaçadas de extinção e que a sua presença tem sido utilizada como indicador da biodiversidade dos ecossistemas florestais. Quanta maior a dimensão de uma árvore, maior é também a biodiversidade que suporta, já que a abundância (quantidade total) e diversidade (número de elementos diferentes existentes) de MAs geralmente aumenta com o PAP. No sudoeste da Europa, particularmente em Portugal, há uma falta de informação sobre a importância das AGP, MAs e biodiversidade associada e que consequentemente leva a uma falta de medidas que ajudem à sua conservação.

Esta dissertação tem como objetivo avaliar a relevância das AGP e seus MAs na conservação da biodiversidade saproxílica (i.e. espécies dependentes de madeira morta) e identificar os fatores que influenciam a formação dos MAs. Para isso, árvores previamente selecionadas foram inventariadas à escala da paisagem no Município de Lousada, Portugal, relativamente à sua diversidade de MAs, a características específicas das árvores e à paisagem (incluindo pressões antropogénicas aplicadas) e micro/macroclima que envolvem a árvore.

Os resultados mostraram que a diversidade de MAs depende de características particulares das árvores, da gestão que lhes é aplicada e de variáveis climáticas. Árvores caducifólias nativas, de grandes dimensões, são as que apresentam uma maior importância para a conservação da biodiversidade saproxílica, uma vez que apresentam maior diversidade de MAs. Foi possível ainda identificar fatores que permitam estimar a diversidade de MAs em árvores, sendo os mais importantes a espécie, PAP e altura. A gestão humana e o tipo de uso do solo são, no entanto, essenciais para explicar a composição de MAs que as árvores têm.

Medidas de preservação das AGP em Portugal são necessárias, tanto a nível nacional como a nível local. Os resultados deste estudo apoiarão o desenvolvimento de estratégias e políticas locais sustentáveis que farão parte do plano de ação local de preservação de AGP em Lousada. É assim dado um primeiro passo na valorização ecológica das AGP em Portugal, esperando que no futuro seja possível replicar estes esforços a escalas espaciais maiores. Identificamos ainda oportunidades de gestão de arvoredos que permitam a preservação a maior escala deste património em Portugal, focando principalmente em leis e regulamentos, abrindo assim novas portas para a valorização que estas árvores tanto precisam.

Palavras chave: Árvores de grande porte, microhabitats, madeira morta, conservação integrativa, indicadores de biodiversidade

ABSTRACT

Human-induced changes that result in the destruction and degradation of ecosystems are leading to dramatic losses in biodiversity at a global scale. Large trees (in our case, those with a trunk with a perimeter at breast height (PBH) larger than 150 cm) have an outstanding social, economic and ecological value. These trees play essential ecological roles in hydrological and micro/mesoclimatic regimes, carbon storage and nutrient cycles, and are also used as habitat for a multitude of living organisms. Large trees have Tree-Related Microhabitats (TreMs) structures like cavities and dead branches that are used for feeding, hiding and nesting for thousands of species. Large TreMs-bearing trees are key elements in the landscape scale, since they can support many endangered species of flora and fauna and have been used as indicators of biodiversity in forest ecosystems. As a tree grows, there is often an increase in its capacity to support biodiversity, since the abundance (total quantity) and diversity (number of different elements) of TreMs usually increase with PBH. In southwestern Europe, particularly in Portugal, there is a lack of information regarding the importance of large trees and their associated biodiversity, which is linked to a lack of regulations to preserve such trees.

This dissertation aims to evaluate the relevance of large trees to promote the conservation of saproxylic biodiversity (i.e. species that depend on deadwood) and identify which factors have a role in the formation of TreMs. For that, a selection of trees was inventoried at a landscape scale in the Lousada Municipality, Portugal, regarding TreMs diversity, tree specific traits and surrounding landscape (including applied management) and micro/macroclimate.

Results showed that TreMs diversity is highly dependent on tree characteristics, applied management and climate variables. Large native deciduous trees are the most important for the conservation of saproxylic biodiversity, having higher TreMs diversity values. Specific tree traits such as the species, PBH and total height can explain a large part of the TreMs diversity in trees and with these data it is possible to estimate the ecological potential of large trees, although it is important to have in mind that human management and land use are important drivers of the TreMs compositions that the trees host.

Large trees preservation measures are necessary in Portugal and should be applied at both national and local level. The results of this study support the development of sustainable local conservation policies that will be part of Lousada’s local conservation action plan. This is a first step taken towards the ecological valorisation of large trees in Portugal and we hope that in the future it will be possible to replicate these efforts in other areas. Further opportunities to extrapolate these efforts at broader scales are highlighted, such as policies and regulations that may trigger this necessary valorisation.

Keywords: Large trees, TreMs, deadwood, integrative conservation, biodiversity indicators

RESUMO ALARGADO

A biodiversidade da Terra está a diminuir a taxas sem precedentes, principalmente pela ação do ser humano levando a alterações climáticas, a poluição, a proliferação de espécies exóticas e ainda a alterações de uso de solo. Este último, que destrói ou degrada ecossistemas, é considerado como uma das maiores ameaças para a perda de biodiversidade e tem alcançado valores nunca antes vistos.

De acordo com a União Internacional para a Conservação da Natureza (IUCN) 27% das espécies avaliadas até hoje encontram-se em risco de extinção. Este desaparecimento de organismos tem impactos na existência humana, afetando não só os recursos que podemos extrair dela, mas também benefícios que nos faculta a nível social, psicológico e cognitivo.

Florestas, um dos ecossistemas mais distribuídos a nível mundial, ocupando cerca de 30.6% da superfície terrestre, são dos ecossistemas terrestres que albergam um maior número de espécies. A nível Europeu ocupa cerca de 33% do território, no entanto apenas 4% se encontra intocada pelo ser humano, e em apenas 13% das restantes é possível encontrar elementos como madeira morta e árvores de grande porte, essenciais à preservação dos organismos saproxílicos que vivem nestes locais e que são extremamente raros em florestas geridas para fins comerciais. Estas florestas intocadas e com elementos essenciais à vida, estão, no entanto, a tornar-se cada vez mais raras uma vez que precisam de longos períodos temporais sem grandes distúrbios para crescer.

Organismos saproxílicos, todos aqueles que dependem de madeira em decomposição de árvores vivas, enfraquecidas ou mortas, representam cerca de um terço dos organismos que vivem nas florestas mundiais. Apesar de terem muitas funções ecológicas diferentes, todos ajudam na decomposição de madeira morta, facilitando o ciclo de nutrientes florestais. O desaparecimento das florestas intocadas ou com madeira morta leva a que grande parte destes organismos se encontre em risco de extinção. Por exemplo, escaravelhos saproxílicos, um dos grupos saproxílicos mais diversos e estudados, tem cerca de 17.9% das espécies europeias avaliadas em risco de extinção, sendo o segundo grupo de invertebrados terrestres europeus com maior número de espécies ameaçadas.

Uma das formas de reverter este declínio generalizado é preservando a madeira morta existente. Esta pode estar disponível não só em árvores mortas, mas também naquelas que estão vivas e albergam nichos de madeira morta com capacidade de albergar um grande número de organismos saproxílicos como cavidades, ramos mortos e feridas. Estes nichos, normalmente denominados de microhabitats que ocorrem em árvores (MAs), podem ser usados como bioindicadores da biodiversidade florestal uma vez que estão ligados à gestão destes espaços, à abundância de madeira morta e a elevados valores de biodiversidade de diversos grupos de organismos.

Árvores de grande porte (AGP) podem ser encontradas em espaços florestais, mas também em campos agrícolas e zonas urbanas. Estas têm sofrido um declínio generalizado por morte natural dos indivíduos existentes mas também por mudanças culturais que levam a alterações de uso de solo onde estas estão integradas, levando ao seu abate. Além disso o facto das pessoas as verem cada vez mais como perigosas para a saúde pública leva a que as entidades públicas as abatam sem fundamentos científicos. No entanto, AGP tanto têm uma simbologia cultural muito forte como são um ecossistema por si mesmas. Estas árvores, quanto maiores forem, maior é a sua capacidade de desempenhar papéis ecológicos essenciais, por exemplos na regulação dos regimes hidrológicos e micro/mesoclimáticos, no armazenamento de carbono e nos ciclos de nutrientes, proporcionando também habitat para uma multiplicidade de seres vivos.

Para inverter as tendências de declínio destas árvores e da madeira morta associada, várias medidas têm sido adotadas a nível europeu, no entanto no sudoeste da Europa, mais propriamente em Portugal, há ainda muito por fazer. Apesar de existirem 81 grupos e 470 indivíduos classificados como monumentais e protegermos duas espécies de árvores em todo o país, continua a faltar uma preservação deste património baseado no seu valor ecológico e uma continua monitorização destas árvores.

Em Lousada, o projeto Gigantes Verdes foi criado em 2017 com o objetivo de identificar e caracterizar as AGP do concelho, utilizando a informação recolhida para criar medidas de conservação dos elementos identificados. Esta dissertação está incluída neste projeto onde pretendo identificar a relevância das AGP e os seus MAs na conservação da biodiversidade saproxílica, utilizando-as como bioindicadores. Para isso, georreferenciamos todas as 7357 árvores de grande porte existentes em Lousada, das quais 2807 foram caracterizadas relativamente à sua diversidade de MAs. Uma vez que é pretendido também identificar os fatores que levam à criação dos diferentes MAs, a diversidade destes foi associado a características das árvores, a variáveis da paisagem e ainda a variáveis micro/macroclimáticas. Este trabalho contribuirá para a integração do conhecimento adquirido na criação e implementação de medidas de gestão das AGP do município de Lousada, Portugal, onde o estudo de caso foi desenvolvido, mas também como uma diretriz para a criação de medidas que possam ser adotadas noutros territórios.

Os resultados mostraram que a diversidade de MAs depende de características das árvores, da gestão que lhes é aplicada e de variáveis climáticas. Árvores caducifólias nativas, de grandes dimensões, são as que apresentam uma maior importância para a conservação da biodiversidade saproxílica, uma vez que apresentam maior diversidade de MAs. Características das árvores tal como a sua espécie, PAP e altura permitem explicar em grande parte a diversidade de MAs presentes nas árvores permitindo que apenas através destes dados seja possível estimar o potencial ecológico das árvores de grande porte, no entanto a gestão humana e o uso do solo são essenciais para explicar a composição de TreMs que as árvores têm. Nas zonas florestais, a grande riqueza de espécies arbóreas leva a que a diversidade de MAs seja extremamente elevada, dado que mais espécies de árvores são capazes de albergar mais organismos nelas, proporcionando uma maior variedade de estruturas para estas utilizarem além de estabelecerem diferentes relações ecológicas. Zonas públicas urbanas encontram-se altamente homogeneizadas em termos de riqueza de espécies de árvores, e dado que são geridas também de forma homogénea, a diversidade de MAs acaba por ser limitada. Além disso, os MAs aparentam estar ligados a grandes amplitudes térmicas. Uma vez que são nichos ecológicos de madeira morta, o aumento das alterações climáticas pode levar a aumentos na criação dessas estruturas, levando à morte de árvores. Espera-se então que as mudanças climáticas futuras tenham um papel na composição das MAs em toda a paisagem, influenciando principalmente as relacionadas à morte dos exemplares.

Medidas de preservação das AGP em Portugal são necessárias, tanto a nível nacional como a nível local. A nível nacional salientamos uma valorização das árvores de grande porte relativamente ao seu valor ecológico, criando mecanismos que permitam preservar árvores relativamente a este papel que elas desempenham. Além disto, um inventário nacional das árvores de grande porte é necessário sendo a base para a conservação destes elementos singulares a nível paisagístico. A nível local, medidas que permitam uma valorização social (aumentando a importância com que estas árvores são vistas pelos seus proprietários) e económica (permitindo ter retornos financeiros que permitam contrabalançar o corte desmesurado de árvores no município) poderão ser um grande passo para a preservação sustentável deste património a largas escalas temporais. Além disso, o município poderá ainda criar mecanismos legais de proteção de alguns exemplares, permitindo uma proteção desde já de árvores com valores ecológicos acima da média.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

SECTION I | GENERAL INTRODUCTION

The Biodiversity Crisis

Biodiversity, *the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems* (Convention on Biological Diversity, 1992) is vital for human's well-being and ecosystems functioning and productivity. From an anthropogenic point of view, biodiversity underlies Nature's Contributions to People (previously designated by Ecosystem Services), i.e., *all the contributions, both positive and negative, of living nature (diversity of organisms, ecosystems, and their associated ecological and evolutionary process) to people's quality of life* (IPBES 2017; Díaz et al. 2018).

In the last decades, there has been a marked decrease of the overall biodiversity suggesting that it is in risk of collapsing (Ceballos et al. 2017) as the outcome of human-induced changes such as climate change, pollution, exotic species proliferation and land use change. The latest, identified as the major driver of biodiversity loss, causes habitat destruction and degradation and has reached unprecedented rates in the human history (Pereira et al. 2012; Titeux et al. 2016; IPBES 2019). According to the International Union for Conservation of Nature (IUCN), "the global authority on the status of the natural world and the measures needed to safeguard it", nowadays, 27% of the world's assessed species are at risk of extinction (IUCN 2019) resulting in the sixth mass biodiversity extinction event in Earth's history (Ceballos et al. 2017). This decline has impacts in the human existence, affecting not only the supply of natural resources but also other psychological, physiological, cognitive, social and spiritual benefits (Keniger et al. 2013).

Forests, one of the widest land use worldwide (accounting for 30.6% of terrestrial land surface (FAO 2018)), are perceived and contribute to humans in different ways, varying according to the intensity of management applied. Naturalized forest, with mature heterogenic stands, are highly valued by the social benefits than can provide contrarily to even-aged monospecific forests that are normally linked to the supply of resources (Ribe 1989; Fairweather and Swaffield 2003). Forests account for 33% of the whole European terrestrial land area and are expanding each year in about 0.33%. Although having a vast area, only 4% of it is untouched and 13% has mature features contrary to the 70% of even-aged forests (Forest Europe 2015; Sabatini et al. 2018). Mature and old-growth forests are references for the assessment of habitat quality and integrity of the overall forest functioning (Kraus and Krumm 2013), yet they are becoming rarer each day due to the absence of the long periods of time without major disturbances that they require to grow. They are rich both in deadwood and large trees, structures generally lacking in managed forests (Lachat and Müller 2018), that are necessary to support and conserve a wide range of threatened species and NCP (Ribe 1989; Bauhus et al. 2009; Seibold et al. 2015; Ikin et al. 2018). Its gradual disappearance is the result of a global afforestation and transformation of forested areas into agricultural or urban areas (Mackey et al. 2015; IPBES 2019).

The Saproxylic Biodiversity

Saproxylic organisms, i.e. those that "*depend, during some part of its life cycle upon wounded or decaying woody material from living, weakened or dead trees*" represent approximately one third of the worldwide forest species and include several taxa groups such as plants, mammals, birds, reptiles, amphibians but mostly insects, fungi and bacteria (Stokland et al. 2012). Saproxylic organisms are a multi-functional group that include wood feeders, fungus feeders, saprophages, parasites, parasitoids, scavengers and predators (Ulyshen 2018) and that are in every trophic level of the forest's food web, ranging from true specialist to generalist species. Although having different functional roles, they all help with deadwood decomposition and consequently promote the nutrient cycles of these ecosystems (Stokland 2012).

For instance, beetles (Insecta, Coleoptera), one of the most diverse and studied groups of saproxylic organisms (Ulyshen 2018), have 17.9% of the assessed European species in risk of extinction, being the second group of terrestrial invertebrates with higher extinction risk in Europe. The major identified threats for these organisms are linked to human-induced changes, with 54.5% of the assessed species suffering from tree age structure gaps, loss of large and old trees, degraded landscapes that are unfriendly to tree growth, and indiscriminate felling for spurious health and safety reasons (Cálix et al. 2018).

Given the particular importance of deadwood for species conservation and nutrient cycles, and also for forest carbon storage, water retention and soil support (Lachat et al. 2013) its preservation and management needs to be improved.

To conserve saproxylic species, deadwood should be available in different quantities and in high structural complex dispositions (Lachat et al. 2013). To do so, thresholds to reach an ecological and sustainable forestry at an European scale have been identified. According to Müller and Bütler (2010) deadwood should be available in quantities that range from 10-150 m^3 per hectare, with peak values around 20-50 m^3, varying between Central-Northern European forest types. Large amounts of deadwood provide higher variety of niches, including more tree species, sizes, diameters, decay classes and positions, increasing its structural complexity and the heterogeneity of habitats, allowing the establishment of more species and the continuity of the saproxylic community (Grove 2002; Lachat et al. 2013). Although thresholds are identified, many countries have been failing to commit to them. In Europe, estimates report an average volume of deadwood in forests of 11.5 m^3/ha, ranging from 0.5 m^3/ha in Albania to 40.6 m^3/ha in Slovak Republic. Furthermore, deadwood is being increasingly acknowledge and valorised in many countries, leading to the adoption of best practice management and an overall increase in its abundance and diversity in recent years (Forest Europe 2015).

Deadwood can be found not only as fallen or standing dead trees (known as snags) and stumps (Humphrey and Bailey 2012), but also in trees that are still alive and are able to bear deadwood ecological niches (known as Tree-Related Microhabitats (TreMs)) such as cavities, bark pockets, large dead branches or trunk rots (Bütler et al. 2013). TreMs have been used as complementary bioindicators of forest sustainability and adopted in forest inventories. They are a more direct predictor of forest biodiversity than deadwood volume (normally used nowadays in Europe) and can link forest management abandonment, abundance of large structural elements and biodiversity for several groups and taxa such has birds, bats and saproxylic beetles (Paillet et al. 2018).

Large Trees and associated TreMs as Biodiversity Indicators

Large trees can be found mainly in forest ecosystems, although they are also found in human-managed ecosystems such as agricultural (Parmain and Bouget 2018; Prevedello et al. 2018) and urban areas (Carpaneto et al. 2010; Treby and Castley 2015; Horák 2018) where they have been recently identified as keystone structures for several taxa (Horák 2018; Parmain and Bouget 2018) but are facing unprecedent threats. The continuously marked decline that large trees face in Europe is mostly an outcome of natural events (e.g. fires, droughts, pests and windstorms) (Lindenmayer and Laurance 2016) and of cultural change. The latter has consequently led to a loss of the symbolic role of these trees and ultimately to 1) the abandonment of traditional land uses, accompanied by an ongoing intensification of modern forestry and agriculture (Bütler et al. 2013) and 2) an increased perception of urban trees as hazardous (Carpaneto et al. 2010) as a result of the associated pollen-related allergies or reactions caused by organisms that live in the tree and of natural felling of leaves, branches and, in some cases, the tree itself (Cariñanos et al. 2017).

Nonetheless, large trees have an ecological importance that is disproportionate to their size (Lindenmayer 2016). With an increase in tree size there is often an increase in 1) carbon storage (Slik et al. 2013; Chen and Luo 2015); 2) hydrologic and micro/meso-climatic regimes control (Nepstad 1994; Dean et al. 1999; Manning et al. 2006; Lindenmayer 2016); 3) nutrient cycles support (Humphrey and Bailey 2012; Lindenmayer and Laurance 2017) and 4) the amount and diversity of TreMs that trees support, providing more habitat for different species (Winter and Möller 2008; Michel and Winter 2009; Vuidot et al. 2011; Larrieu and Cabanettes 2012; Bütler et al. 2013; Regnery et al. 2013; Bouget et al. 2014; Johann and Schaich 2016; Abdullah et al. 2017; Großmann et al. 2018; Kraus et al. 2018; Asbeck et al. 2019). Furthermore, large trees also act as legacies of biological continuity i.e. they sustain biological and ecological continuity of genetic resources and habitats for a variety of organisms, including endangered, specialized, rare and threatened ones (Alexander and Tree 2008; Bütler et al. 2013), even if the surrounding habitat has changed profoundly (Hunter et al. 2016). Likewise, large trees are indicators of past climatic conditions (Babst et al. 2014; Young et al. 2015; Moga et al. 2016)) and are a vital part of socio-economic systems, being linked to people by historical, cultural, aesthetic and spiritual values and by bearing information on human management interventions and traditional land uses (Marques 1987; Blicharska et al. 2013; Babst et al. 2014; Blicharska and Mikusinski 2014; Moga et al. 2016; Lopes et al. 2019).

To mitigate the impacts of the lack of deadwood and large trees in managed forests, the retention of these structures is being identified and prioritized as a key biodiversity conservation action to be adopted (Cálix et al. 2018) and implemented throughout Europe (Kraus and Krumm 2013; Hagge et al. 2018; Mölder et al. 2019). Prévot-Julliard et al. (2011) are some of the many that suggest that a co-existence between humans and nature is necessary to alter the biodiversity crisis, and that implementing integrative conservation strategies can complement the segregative strategy that led to the implementation of worldwide protected areas, allowing to fix some of the problems that these areas have in protecting species (Bosso et al. 2017; Rada et al. 2019). It is suggested that high-quality diverse ecosystems should be preserved, yes, but a balance of people needs and ecological resources exploitation by remediating impaired systems and increasing the appreciation that humans have regarding nature is necessary (Prévot-Julliard et al. 2011).

In recent years several measures have been discussed and implemented to preserve and valuate large trees worldwide, in a great part, by creating social and economic benefits to the people who own them. Those measures can be e.g. the identification and protection/retention of single and grouped large trees of particular ecologic and cultural interest (Blicharska and Angelstam 2010; Bäuerle and Nothdurft 2011; Franklin and Johnson 2012; Gustafsson et al. 2012; Kraus and Krumm 2013; Blicharska and Mikusinski 2014; Lindenmayer 2016; Moga et al. 2016), to the creation of methods to diminish the mortality of existing large trees and promote the long term grow of smaller trees (Manning et al. 2013; Le Roux et al. 2014; Lindenmayer and Laurance 2017), but also the creation of artificial structures that allow a short-term compensation of habitat resources (Le Roux et al. 2014, 2016). Furthermore, measures to increase the awareness about the importance of large trees at a global scale have been implemented (Blicharska and Mikusinski 2014) and the creation of economic incentives for the protection of such trees at local and national levels have been adopted (Cullen 2007; Blicharska and Mikusinski 2014; Doick et al. 2018).

Portugal, A Forestry Country

Forested areas in Portugal represent 36% of the territory and are nowadays the main land use in the country (ICNF 2019). Major transformations in land use have happened in the country in the last 30 year, triggered mainly by socio-economic growth and development (Jones et al. 2011; Meneses et al. 2017). During this period, urbanization which occurred mostly in the northwest and south of Portugal, fruit of historical reasons and absence or limited application of land use planning (Freire et al. 2009) resulted in an expansion of 38.8% of the urban areas (ICNF 2019). Agricultural areas decreased about 13% during this same period (ICNF 2019), with small agriculture in the north being mostly abandoned, replaced by shrublands or transformed into *Eucalyptus globulus* plantations (Meneses et al. 2017; Deus et al. 2018). In the Alentejo region (centre-south) agriculture land transformation, e.g. olive and almond plantations intensification, derived mainly from the construction of dams that allowed the creation of more intensive methods in place of traditional ones (Jones et al. 2011; Meneses et al. 2017). The centre of Portugal was mostly transformed regarding its forests, with coniferous trees being substituted by *E. globulus* plantations by small private owners and cellulose companies (Meneses et al. 2017; Deus et al. 2018). Forests had a generalized increase through the last decades, despite light fluctuations of growth along the years (ICNF 2019). This is mostly at the expenses of an increment in monoculture plantations of *E. globulus (*between 1970-2010 increased more than 400% (Deus et al. 2018)) that nowadays account with 26% of the forested areas in Portugal (ICNF 2019). These increase of dense monocultural forests have two major effects: 1) they lead to a generalised decrease of biodiversity (Matos 2011; Deus et al. 2018) and 2) they favour the development of high-intensity fires, especially if they are found in large continuous landscapes divided in small private areas that are poorly managed (Fernandes et al. 2016; Gómez-González et al. 2018). In 2017, one of the worst years in terms of forest fires in Portugal (burning 442.418 forest hectares (ICNF 2017) and killing 109 persons (Comissão Técnica Independente 2017, 2018)), an existing (but dormant) forest cleaning law (Law nº124/2006) was updated and implemented at large scale (Law nº76/2017) with the purpose to prevent new events like the previous ones of happening again. This law implemented large-scale fines for those who would not comply with the cleaning of a buffer area (10-50 metres) surrounding buildings and roads. Consequently, it led to a national scale arbitrary removal of deadwood and finer debris from forests and to a cut of large trees in the buffer areas, mostly as an outcome of the lack of knowledge of the law by citizens and a generalised fear of the fines and fires.

In 2015, Portugal only had an average of 2.7 m^3/ha of deadwood available in its forests and exclusively standing (Forest Europe 2015), since most of the fallen deadwood is culturally removed for firewood. With the implementation of the forest cleaning law, it is expected that deadwood abundance might decrease in a small and long term. Within this context, in a country that does not acknowledge the importance of deadwood, and its clearance is favoured in national forestry law, how can saproxylic organisms survive? And how can we help preserve them? It is not easy but is also not impossible and the preservation of living large trees and their deadwood ecological niches (the TreMs) might be one of the answers. The protection of these trees can have a significant impact in overall saproxylic species conservation (Jonsell 2012), although not substituting fallen or standing dead trees, since they harbour different species communities (Bouget et al. 2012).

Portugal has 81 arboreal groups and 470 singular trees protected and classified as of public interest and consequently as monumental (Lopes et al. 2019). The national classification system recognises a tree as monumental if it stands out regarding exceptional characteristics such as size, structure, age, rarity or if it has a significative natural, historical, cultural or aesthetic value (Law nº53/2012, that updates the Decree-Law nº 28/468 of 1938, and is further regulated by Ordinance nº. 124/2014). Although it comprises a multiplicity of features that allow a generalised classification and protection of trees, none of them comprises the exceptional value that trees might have for nature conservation. Around the world several classifications that include an ecological value have been adopted and its use helps to acknowledge its value. The most broadly used terms are veteran, ancient or habitat tree (very large and/or very old, living or dead in the case of habitat-tree) microhabitat-bearing trees that are of prime importance for specialised forest flora and fauna tree (Woodland Trust 2008; Bütler et al. 2013)). Furthermore, Portugal also has laws for the protection of two tree species (*Quercus suber* and *Q. ilex)* (Decree-Law 155/2004), allowing them to grow to become large, although, when dead, they can be removed from the landscape. Although protected, there is no inventory of how many and where the specimens of these two species are distributed (at a tree level) throughout the country, leading to a consequent lack of monitoring.

The first concrete steps towards deadwood, TreMs-bearing trees and saproxylic organisms conservation in Portugal were made in 2016 when the VACALOURA.pt project started (Soutinho et al. 2017). VACALOURA.pt is a voluntary based project that aims to conserve the European-Stag-Beetle (ESB), *Lucanus cervus*, in Portugal. This near-threatened protected species is linked to citizens and it is used as a flagship species of saproxylic organisms (Campanaro et al. 2016).

The project has been able to reach citizens and entities that are concerned about the disappearance of the ESB in Portugal. VACALOURA.pt uses citizen science to compile information regarding the species distribution and population trends throughout the country, while promoting environmental education activities and workshops for all ages. The results achieved so far have been allowing the project to implement national and local scale deadwood and large trees management strategies while working with public and private decision makers. By including citizens in the project, it aims to create also a social impact and valorisation of the species and its habitats. In only 2 years of data collecting, an increase of 36% in terms of species distribution area was achieved (Soutinho et al. 2017) and recent reports shows increases of more than 50% in only 4 years. This is an example of the lack of information that exists in Portugal regarding saproxylic organisms, that consequently results in a lack of conservation measures towards its habitats.

The Lousada Case Study

Lousada municipality is a perfect example of the national reality in terms of land-use occupancy and its ongoing changes, especially in the temperate northwest of Portugal. With a density of 493.2 habitants/km^2 (national average of 110 habitants/km^2) (INE 2011) it has a highly diversified landscape (Abrantes et al. 2018) dominated by forests and shrubland (44%, with *E. globulus* areas representing 61% of it), agricultural areas (36%) and urban areas (20%) (DGT 2018). Lousada's major land use changes are linked to the increasing urban areas over agricultural and forested areas (Abrantes et al. 2018). Large trees are cut at a 3% yearly rate, with increased urbanization being the main known cause of clearance (40% of the cut trees), followed by agriculture intensification (17%), private personal management (12%) and public safety (11%) with natural death only accounting for 5% of the cutting causes (own data). For 15% of the cut trees was impossible to assess the reason for clearance. When extrapolating for 20 years ahead we can expect a decrease of more than 45% of these large existing trees.

Despite this reality, Lousada's municipality has been developing an extraordinary sustainability strategy that aims to develop different environmental education and nature conservation projects in the municipality, aiming to diminish future problems by giving tools to citizens to decide their own faith, being an example of integrative conservation. This strategy is focused on five axes 1) environmental education and scientific outreach, 2) research and nature conservation, 3) societal engagement, 4) infrastructural actions and 5) internal sustainability.

The municipality understands that to achieve significant goals in terms of nature conservation, the participation and involvement of local communities is necessary. This strategy builds upon the idea that nature conservation and environmental education should be interlinked, and the outcome of its implementation is an increased public perception and collective environmental awareness. The axes of this strategy are interdependent and necessary to implement the projects that are running nowadays.

Along the years, several projects have been created by the municipality to link people to nature to improve nature conservation. It all started with a large-scale inventory of the natural patrimony of the municipality back in 2014, and nowadays the ongoing projects tackle environmental education done in schools (with more than 16000 students reached in 2018-2019) and to families (with more than 1000 people reached yearly) regarding conservation of rivers and terrestrial habitats. Citizens are being accountable for their future and with voluntary community work was possible to plant more than 40000 native trees and to monitor and recover more than 10km of river margins in the last four years. Large temporal and spatial scale integrative conservation measures were created to improve agriculture and forest private areas. The municipality is also creating local protected areas to promote the valorisation of local and cultural methods that link social, economic and nature preservation.

Regarding the previously question *"in a country that does not acknowledge the importance of deadwood, how can saproxylic organisms survive? And how can we help preserve them?"* Lousada is actually leading a pilot project to develop some answers. Firstly, a 14 hectares native forest, formerly privately owned, was acquired by the municipality and is nowadays being managed to promote nature conservation, with active increment of deadwood as one of its main tools. The deadwood comes from trees that naturally felled or were cut for safety reasons but also from branches resulting from public trees pruning. They are displayed as heterogenic as possible although respecting some rules since this display has the double purpose of create a multitude of habitats and improve the public use of the space by visitors, e.g. by limiting their access to important preservation areas (Figure 1.1 and 1.2). Furthermore, the Gigantes Verdes (Green Giants) project was created in 2017 to inventory the large trees (with a perimeter at breast height higher than 150 cm) of the municipality and show its importance for the conservation of the biodiversity that depends on them. This project has the goal to promote evidence-based management criteria to support the protection of such large trees within the municipality and hopefully, in the future, throughout the country. The compiled data will allow the creation of a social, economic and ecological valorisation of this patrimony throughout the municipality as a pathway to support its preservation on a long run (Figure 1.3).

Figure 1.1 - Tree that naturally felled in the forest and which limbs were removed for safety reasons regarding visitors. Limbs and branches were used to create other deadwood structures.

Figure 1.2 - Trees that were cut for safety reasons and were transported to the forest, serving as benches for visitors

In this context, this dissertation aims to identify how large trees promote the conservation of saproxylic biodiversity at a landscape level. For that, we evaluated large trees regarding their TreMs diversity and compositions and linked the results with tree traits and surrounding landscape (including applied management) and micro/macroclimate variables with the aim to build explanatory and predictive models. This work will contribute to the integration of evidence-based knowledge into the design of large trees management plan at the municipality level of Lousada, Portugal, where the case study was developed, but also introduce guidelines to support the protection of saproxylic biodiversity for other municipalities.

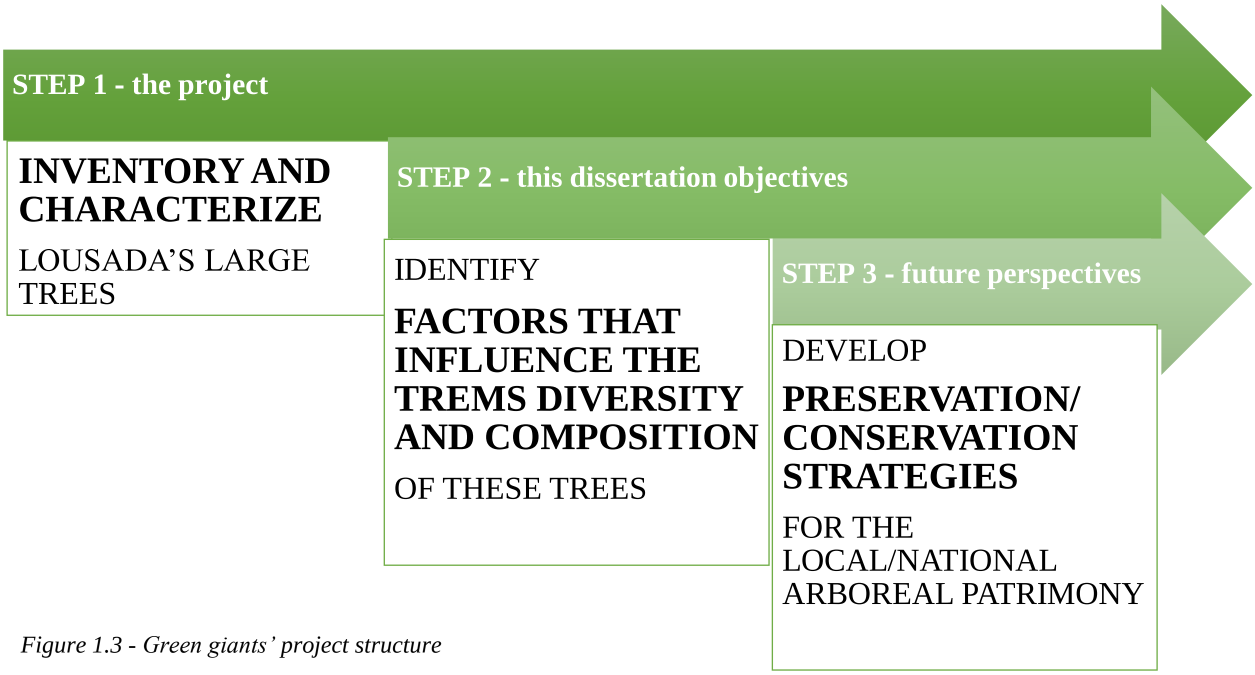

Figure 1.3 - Green giants' project structure

Dissertation Structure

This dissertation is organized into three major sections. The first presents a general introduction about the theme and aims of the dissertation. The second section, presented as a scientific article, aims to identify characteristics at the tree, landscape and micro/macroclimate level that are linked to high diversity of TreMs and consequently high saproxylic biodiversity in the Lousada's large trees. The main findings of this section are summarized and discussed in the latest section of the dissertation with a focus of how this data can be translated into action management plans to preserve these trees.

SECTION II | CASE STUDY

"To cut or not to cut. The importance of conserving large trees for maintaining saproxylic diversity in southwestern Europe"

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Abstract
Earth’s biodiversity is facing dramatic losses due to human-induced changes that lead to destruction and degradation of ecosystems. Large trees (in our case, those with a trunk with a perimeter at breast height (PBH) larger than 150 cm) can be both a cultural symbol and an ecosystem themselves. These trees play essential ecological roles in hydrological and micro/mesoclimatic regimes, carbon storage and nutrient cycles, and are also used as habitat by a multitude of living organisms. Large trees support structures such as cavities and dead branches, known as Tree-Related Microhabitats (TreMs) that are places for feeding, hiding and nesting for thousands of species. Large TreMs-bearing trees are keystone elements at the landscape scale, since they can support many species, including endangered flora and fauna.

This study aims to evaluate the relevance of large trees to promote the conservation of saproxylic biodiversity at a landscape scale and identify which factors have a role in TreMs formation. For that, a selection of trees was inventoried at a landscape scale in the Lousada Municipality, Portugal, regarding their TreMs diversity, tree specific traits and surrounding landscape and micro/macroclimate.

Results showed that TreMs diversity is highly dependent on tree characteristics, applied management and climate variables. Large native deciduous trees are the most important for the conservation of saproxylic biodiversity, since are the ones with higher TreMs diversity. Specific tree traits such as its species, PBH and total height can explain a large part of the TreMs diversity in trees and with these data it is possible to estimate the ecological potential of large trees, although it is important to have in mind that human management and land use are important drivers of the TreMs compositions that trees host.

Large trees conservation measures are necessary in Portugal and should be applied at both national and local levels. The results of this study support the development of sustainable local conservation policies and guidelines that will be part of Lousada’s local conservation action plan. This is a first step taken towards the ecological valorisation of large trees in Portugal and we hope that in the future these efforts replicated in other areas.

Keywords: Large trees, TreMs, deadwood, integrative conservation, biodiversity indicator

Introduction

Earth's biodiversity is facing dramatic losses due to human-induced changes such as e.g. climate change, pollution, invasive species and land-use change. The latter, identified as the major driver of biodiversity loss, causes habitat destruction and degradation and has reached unprecedented rates in the human history (Pereira et al. 2012; Titeux et al. 2016; IPBES 2019). Most of the increase in urbanization and agricultural expansion, has come at the expense of forest, particularly of old-growth forests that are becoming very rare (Sabatini et al. 2018).

Old-growth forests require a long time to grow and are extremely rich in large trees and the associated large amounts of deadwood, both necessary to support and conserve a wide range of species and ecosystems functioning (Bauhus et al. 2009; Ikin et al. 2018). Besides forest areas, large trees can also be found in other human-managed ecosystems such as agricultural (Parmain and Bouget 2018; Prevedello et al. 2018) or urban areas (Carpaneto et al. 2010; Treby and Castley 2015; Horák 2018) where have been identified as keystone elements for the conservation of biodiversity. The continuously marked decline that large trees face in Europe is an outcome of natural events (e.g., fires, droughts, pests and windstorms) (Lindenmayer and Laurance 2016) and of cultural change. This last has consequently led to a loss of the symbolic role of large trees and ultimately to 1) the abandonment of traditional land uses, accompanied by an ongoing intensification of modern forestry and agriculture (Bütler et al. 2013) and 2) an increased perception of urban trees as hazardous (Carpaneto et al. 2010), either because of associated pollen-related allergies or reactions caused by organisms that live in trees, or because the natural felling of leaves, branches and, in some cases, the tree itself (Cariñanos et al. 2017).

Large trees are vital to biodiversity because they act as long-term legacies of biological continuity, i.e. they sustain biological and ecological permanency of genetic resources and habitats for a myriad of organisms, including endangered, specialized, rare and threatened ones (Alexander and Tree 2008; Bütler et al. 2013). The importance of large trees is observed even when the tree is isolated, and the surrounding matrix shows environmentally impacted (Hunter et al. 2016). Likewise, old-trees can help us understand past climatic conditions (Babst et al. 2014; Moga et al. 2016) and are a vital part of socio-economic systems being linked to people by their historical, cultural, aesthetic and spiritual values and by bearing information on human management interventions and traditional land uses (Marques 1987; Blicharska et al. 2013; Babst et al. 2014; Blicharska and Mikusinski 2014; Moga et al. 2016; Lopes et al. 2019). Large trees have also an ecological importance that is disproportionate to their scale when considering the landscape (Lindenmayer 2016). With an increasing tree size there is often an increase in 1) carbon storage (Slik et al. 2013; Chen and Luo 2015), 2) regulation of hydrologic and micro/meso-climatic regimes (Nepstad 1994; Dean et al. 1999; Manning et al. 2006; Lindenmayer 2016), 3) support of nutrient cycles (Humphrey and Bailey 2012; Lindenmayer and Laurance 2017) and 4) increase of the amount and diversity of Tree Related Microhabitats (TreMs) (Winter and Möller 2008; Michel and Winter 2009; Ragón et al. 2010; Vuidot et al. 2011; Larrieu and Cabanettes 2012; Regnery et al. 2013; Bütler et al. 2013; Bouget et al. 2014; Johann and Schaich 2016; Abdullah et al. 2017; Großmann et al. 2018; Kraus et al. 2018; Asbeck et al. 2019). TreMs are all the tree cavities, bark pockets, large dead branches, trunk rots or deadwood (Bütler et al. 2013). Altogether they provide habitat for a specific and unique type of biodiversity - saproxylic organisms - and can be used as indicators of forest biodiversity. In some cases, it is shown that TreMs can be related with past management practices, microclimate, or allow to predict the presence of potential taxonomic groups (Paillet et al. 2018).

Saproxylic organisms, which are "dependent, during some part of its life cycle, upon wounded or decaying woody material from living, weakened or dead trees", represent approximately one third of the worldwide forest species and include several taxa groups such as plants, mammals, birds, reptiles, amphibians, but mostly insects, fungi and bacteria (Stokland et al. 2012). In particular, beetles (Insecta, Coleoptera), one of the most diverse groups of saproxylic organisms, face serious threats at the European level (Seibold et al. 2015). The last report on the extinction risk of these organisms in Europe showed that 17.9% of the assessed species are at risk. The major identified threats relate with human-induced changes; 54.5% of the assessed species are being constrained from tree age structure gaps, loss of large and old trees, degraded landscapes that are unfriendly to tree growth, and indiscriminate felling for spurious health and safety reasons (Cálix et al. 2018). The importance of deadwood is increasingly acknowledged in many countries, leading to the adoption of better forest management and overall promotion of its abundance and diversity (Forest Europe 2015). Since a large number of species depend exclusively on large trees and their deadwood to survive, their large-scale preservation is identified as a key biodiversity conservation action to be adopted (Cálix et al. 2018).

In countries where the importance of deadwood is not yet acknowledged, the preservation of living large trees that bear TreMs and that are not removed by humans is crucial. This situation is aggravated by the common management practice such as the fast removal of dead trees from the field. Thus, the protection of these trees can have a significant impact in overall saproxylic species conservation (Jonsell 2012), although not substituting fallen or standing dead trees, since they harbour different communities of organisms (Bouget et al. 2012).

The acknowledgement of the importance of large trees and of deadwood has already been recognized in some European countries such as Germany and France and their abundance and diversity are increasing as the result of conservation actions implementation. However, Southwestern Europe is still giving the first steps in recognizing the importance of large trees and of deadwood. For Spain, no data was found regarding deadwood abundance and its management to maintain biodiversity although some scientific research has been developed in the last decade (e.g., Iglesias 2009; Micó et al. 2010; Ramilo et al. 2017). For Portuguese forests, only 2.7 m3/ha of deadwood has been reported (Forest Europe 2015), and so far, there are no regulations to manage or study it. Thus, a lot needs to be done to reach suggested thresholds for deadwood in other European forests, e.g., that range from 10-50 m3/ha (Müller and Bütler, 2010), or the European average of deadwood which is 11,5 m3/ha (Forest Europe 2015).

The protection of large trees is associated with local laws and the characteristics of the trees that are most valued. In Portugal, for example, two species of trees (*Quercus suber* and *Q. ilex*) are protected by national law, limiting the management that can be applied towards the existent individuals. However, there is any tree-level inventory on how many trees and its locations at the country level. For Portugal, only trees classified as monumental and therefore of public national interest are conserved by law (although the Portuguese classification system does not acknowledge their importance for nature conservation). In this case there's detailed knowledge and there are 470 singular trees and 81 arboreal groups of public interest that are protected in the whole country level (Lopes et al. 2019).

In recent years several measures have been discussed and implemented to preserve large trees worldwide. They range from the identification and protection/retention of single and grouped large trees of particular ecologic and cultural interest (Blicharska and Angelstam 2010; Bäuerle and Nothdurft 2011; Franklin and Johnson 2012; Gustafsson et al. 2012; Kraus and Krumm 2013; Blicharska and Mikusinski 2014; Lindenmayer 2016; Moga et al. 2016; Cálix et al. 2018) to create methods to diminish the mortality of existing large trees and promote the long term growth of smaller trees (Manning et al. 2013; Le Roux et al. 2014; Lindenmayer and Laurance 2017), but also to create artificial structures that allow a short-term compensation of this habitat's resources (Le Roux et al. 2014, 2016). Also, it includes measures to increase public awareness about the importance of large trees and associated deadwood at a global scale (Blicharska and Mikusinski 2014) and create economic incentives for the protection of such trees at local and national levels (Cullen 2007; Blicharska and Mikusinski 2014; Doick et al. 2018).

In this study, we aim to identify how large trees promote the conservation of saproxylic biodiversity at a landscape scale. For that, large trees were evaluated regarding their TreMs diversity and compositions. Further, and as a mean to understand the factors behind TreMs formation, tree traits and surrounding landscape (including applied management) and micro/macroclimate variables were also inventoried with the aim to build explanatory and predictive models. This work will contribute to the integration of evidence-based knowledge into the design of large trees management plan at the municipality level of

Lousada, Portugal, where the case study was developed, but also introduce guidelines to support the protection of large trees for other municipalities.

Methods

Study area

The work took place at the Municipality of Lousada (N 41.27711º, W 8.28290º), located in Porto District, Portugal (Figure 2.1). This municipality has an area of 96.08 km^2 and a density of 493.2 habitants/km^2 (INE 2011). The climate is classified as in a Temperate Bioclimatic zone with moderated temperatures in summer and winter, with an annual thermic amplitude of 12ºC (Monteiro 2005). This municipality is characterized by a landscape mosaic dominated by forest or other "natural or semi-natural" areas (44%), agricultural (36%) and urban areas (20%) (DGT 2018). Regarding tree species protection, except for the specimens of cork oak (*Quercus suber)* that are protected at the national level, there are no other protected trees in Lousada (Lopes et al. 2019) or even local policy to protect this natural patrimony. The present study is part of the project GIGANTES VERDES (green giants) which aims to identify and characterize the existing large trees of the municipality. With this information the municipality intends to define local management practices and conservation criteria for large trees and introduce guidelines for the conservation of large trees' in Portugal.

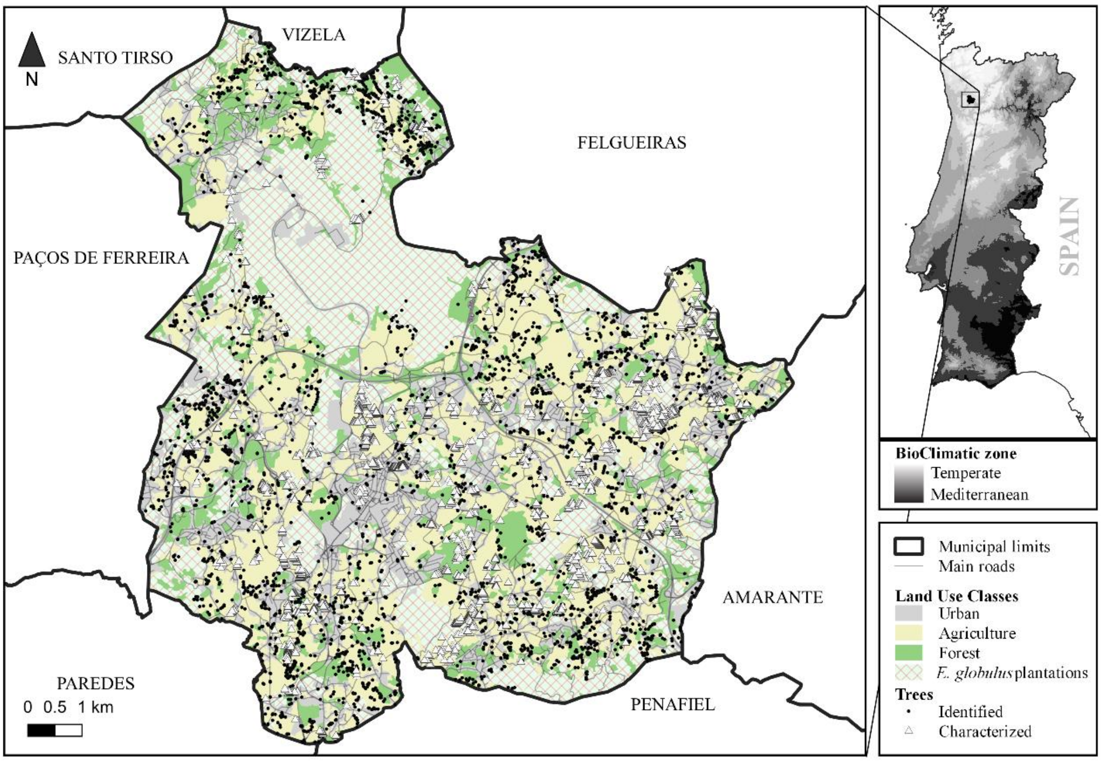


Figure 2.1 - Sampling area and distribution of the trees identified and characterized by land use in Lousada municipality, Portugal. The map of Portugal shows the macrobioclim variations with the country, ranging from temperate (white) to Mediterranean areas (black).

Sampling

Sampling was carried out in two phases: 1) extensive georeferentiation of the large trees (those with a perimeter at breast height (PBH) > 150 cm) of Lousada by direct observation between November 2017 and December 2018, and 2) thorough characterization of large trees regarding the diversity of TreMs (number of different TreMs that are available in the tree), tree specific traits, management history and macro/microclimate variables (Table 2.1) between January and July 2019. TreMs were classified using the catalogue of tree microhabitats developed by Kraus et al (2016) (Supplementary material 1). The tree selection was stratified at the land-use level considering the following three classes 1) urban, 2) agriculture and 3) forest, according to the 2015 Portuguese land use cartography (DGT 2018). *Eucalyptus globulus* plantation areas (61% of Lousada's forested area) were excluded from sampling since these areas are clear-cut frequently during forestry procedures. However, if any large tree was sighted in those stands, it was identified. The sampling methodology was based on the characterization of land-use classes where they could be found potentially, such as forests, agriculture and urban areas. Then tree samples were stratified at the three land-use types. The specific sampling sites within each land type were selected randomly. Sites which were not easily accessed (private areas without access) were discarded from the sampling and the next random sampling point was then selected. The final location of the sampling that occurred is depicted in Figure 2.1.

Data analyses

Two different analysis were carried out since we were interested in understanding how the group of analysed variables influence the TreMs composition of each tree but also in identifying which variables mostly influence the diversity of TreMs in trees at a landscape scale. For that, and starting at the individual tree analysis, non-metric multidimensional scaling (NMDS) ordination was performed based on a matrix where, for each tree, its species identity and TreMs category richness (number of available TreMs of each TreMs category) were used, in order to group the trees regarding their most similar TreMs compositions (the group of available TreMs). The best solution was run with Bray-Curtis distance (McCune et al. 2002), chosen from 40 runs, each starting randomly. Relationships between the NMDS ordination and tree traits, management and environmental variables (including micro and macroclimatic) were examined by fitting vectors to the ordination plots (McCune et al. 2002) using the 'envfit' function of the 'vegan' package (Oksanen et al., 2005). For the analysis, we considered tree species with more than 80 specimens, remaining therefore with the ten most common ones.

To understand the broader relationships that dictate TreMs diversity in trees, Generalized Linear Models (GLM) with a Poisson error distribution for count data were used. The models included the variables which 1) showed significant correlations ($p<0.05$) in the NMDS (Table 2.2), 2) were not correlated among them and 3) did not have any multicollinearity. For the correlation analysis, we used Pearson correlation tests for numerical variables considering significance for $p<0.05$ and a cut-off of correlation >70%. For categorical variables, Chi-squared tests were used to check the association between the variables. Again, signification was considered for $p<0.05$. To check for collinearity, Variance Inflation Factors (VIFs), which supports modelling adequacy were used with a cut-off of VIF>10. Regarding tree traits, two levels of information were used to create GLM. The first (Model 1 – Table 2.3) included species/genus level information to measure TreMs diversity and the second (Model 2 – Table 2.3) allows a broader analysis by only differentiating trees regarding their origin and physiology.

In order to create predictive simplified models, we used the 'dredge' function from the 'MuMIn' package (Barton 2019) to identify the best model with only 3 variables based on the previous explanatory Model 1 (Model 3 – Table 2.3).

For the creation of the models, outliers were eliminated based on Cook distances > 0.005. The models were later validated using the Pearson test to correlate the modelled values with the real ones for each tree (Zheng and Agresti 2000; Elith et al. 2006).

The data analyses were conducted using RStudio software Version 1.1.463 (R Core Team 2017), the graphics created used ggplot2 plugin (Wickham 2016) and geospatial analyses were executed using QGis v3.4.1-Madeira (QGis Developing Team 2019).

Table 2.1 - Characterization of the variables measured at the tree and landscape levels in Lousada, Portugal including methods of acquisition (n=2873 trees). For categorical variables, an ordination value regarding the intensity of the factor was created so that tendencies might be observed in the ordination plot (Ord.val).

	CODE	VARIABLE	DETAILS INCLUDING METHODS OF ACQUISITION	Ord.val	Variable range (% in the sample)	Median	Mean (S.D)
Tree Traits	*TreMs*	*Tree related microhabitats diversity*	Identification of presence/absence of each of the 64 Tree-Related Microhabitats from the Catalogue of Microhabitats (Kraus et al. 2016)		*0 - 27*	8	8.2 (3.5)
	Sp	*Species*	Identification at the species level (when not possible to Genus level) accordingly to the information available at the Flora.On project website (Flora de Portugal Interativa, 2014)		*56 Species/Genus*	--	--
	hlt	*Health*	The state of health of the tree, separating dead from alive if no sign of vitality was found.	0 1	*Dead (0.6%)* *Alive (99.4%)*	--	--
	Or	*Origin*	Distinction between species that occur naturally in Portugal (native) or that were introduced to our Flora. Information for each species was collected accordingly to the information available at the Flora.On project website (Flora de Portugal Interativa, 2014)	0 1	*Exotic (57%)* *Native (43%)*	--	--
	Ph	*Physiology*	Distinction between the physiology of the species, separating Angiosperms of Gymnosperms. Information for each species was collected accordingly to the information available at the Flora.On project website (Flora de Portugal Interativa, 2014)	0 1	*Gimnosperm (16%)* *Angiosperm (84%)*	--	--
	PBH	*Perimeter at breast height (cm)*	The measure of the PBH for each tree was carried with Haglof Mantax Blue 1m Caliper (that measures the diameter at breast height and that it was converted to perimeter by multiplying for π). When the tree was too big, perimeter was measured with a measuring tape at breast height.		*47.7 - 226*	62	65.8 (15.6)
	Hg	*Tree total height (m)*	The measure of the height for each tree using a Clinometer HagLof ECII		*2.8 - 46.4*	20	20.1 (7.3)
Tree Management	*Prop*	*Property*	Distinction between public or privately-owned trees based on the ownership of the area where it is growing.	0 1	*Private (73%)* *Public (27%)*	--	--
	Group	*Grouping*	Evaluation of the surrounding environment and identification of the grouping category that the tree is involved	0 1 2	*Isolated (8%)* *Aligned (25%)* *Grouped (67%)*	--	--
	Pr.freq	*Pruning frequency*	Identification of the pruning frequency based on trees that are actively managed at a periodic scale, trees that have signs of arbitrary pruning or no signs	0 1 2	*No signal (59%)* *Arbitrary (35%)* *Periodic (6%)*	--	--
	Pr.qual	*Types of wrong cuts from pruning*	A badly executed pruning might leave marks on the tree such has wrong cuts (to close to the tree trunk or too far away, leaving canopy deadwood) or even debarking when no safety cuts are executed. In this category, for each tree, the diversity of wrong cuts where identified, ranging from 0 to 3, being the maximum a tree with close cuts, deadwood from long cuts and debarking.	0 1 2 3	*0 (63%)* *1 (11%)* *2 (15%)* *3 (11%)*	--	--
	LU.tg	*Land use where the tree is growing*	Based on the first level of detail of the Land Use Cartography from Portugal (DGT 2019) was possible to identify the land use where the tree is growing.	1 2 3	*Urban (16%)* *Forest (62%)* *Agriculture (22%)*	--	--
	LU.urb	*The surrounding area of urban land use (m2)*	Based on the first level of detail of the Land Use Cartography from Portugal (DGT 2019). A 50-meter buffer was created where the area within the buffer of urban, agricultural and forested land use was calculated for each tree.		*0 - 7725*	0	1256 (2382)
	LU.agr	*The surrounding area of agriculture land use (m2)*			*0 - 7725*	2416	2825 (2595)

LU.for	*The surrounding area of forest land use (m2)*		*0 - 7725*	3979	3643 (2804)

	CODE	VARIABLE	DETAILS INCLUDING METHODS OF ACQUISITION	Variable range	Median	Mean (S.D)
macroclimate	*Arid*	*Global Aridity Index*	Data extracted from the Global Aridity Index and Potential Evapotranspiration Climate Database v2 (Trabuco and Robert 2019), available at https://cgiarcsi.community/2019/01/24/global-aridity-index-and-potential-evapotranspiration-climate-database-v2/. Raster cells have approximately 600m^2 of area and data was extrapolated for each tree by intersection of the tree point with the raster layer, resulting the value of the raster cell where the tree is.	*10809-11289*	10893	10956 (121)
	EvTr	*Global Reference Evapo-Transpiration (mm/day)*		*1212 - 1244*	1224	1224 (6)
	Elev	*Elevation*	Land elevation data based on a 250m^2 raster extracted from http://www.dgterritorio.pt/dados_abertos/mdt/	*162 - 498*	220.50	237.96 (58.69)
	Bio1	*Annual Mean Temperature (ºC)*		*13.14 – 14.37*	14.19	14.12 (0.22)
	Bio2	*Mean Diurnal Range (ºC)*		*9.72 – 10.49*	10.23	10.20 (0.17)
	Bio3	*Isothermality (ºC)*		*42.48 – 45.46*	44.45	44.42 (0.55)
	Bio4	*Temp. Seasonality (ºC)*		*452.65 – 482.46*	462.90	463.64 (5.01)
	Bio5	*Max Temp. of Warmest Month (ºC)*		*24.13 – 25.60*	25.26	25.24 (0.26)
	Bio6	*Min Temp. of Coldest Month (ºC)*		*1.23 – 2.88*	2.24	2.27 (0.31)
	Bio7	*Temp. Annual Range (ºC)*		*22.50 – 23.24*	23.00	22.96 (0.15)
	Bio8	*Mean Temp. of Wettest Quarter (ºC)*		*7.64 – 9.05*	8.80	8.74 (0.25)
	Bio9	*Mean Temp. of Driest Quarter (ºC)*	Data extracted from the WorldClim – Global Climate Data (Fick and Hijmans 2017) available at http://worldclim.org/bioclim. Raster cells have approximately 1 km^2 in area and data was extrapolated for each tree by the intersection of the tree point with the raster layer, resulting in the value of the raster cell where the tree is.	*19.29 – 20.17*	20.02	19.98 (0.16)
	Bio10	*Mean Temp. of Warmest Quarter (ºC)*		*19.35 – 20.27*	20.12	20.09 (0.16)
	Bio11	*Mean Temp.of Coldest Quarter (ºC)*		*7.64 – 9.05*	8.80	8.74 (0.25)
	Bio12	*Annual Precipitation (mm)*		*1321.95 – 1395.11*	1336.04	1341.96 (16.37)
	Bio13	*Prec.of Wettest Month (mm)*		*195.86 – 201.13*	198.00	197.87 (0.87)
	Bio14	*Prec. of Driest Month (mm)*		*18.00 – 20.00*	19.00	18.89 (0.37)
	Bio15	*Prec. Seasonality (mm)*		*53.57 – 54.53*	54.12	54.12 (0.2)
	Bio16	*Prec of Wettest Quarter (mm)*		*548.05 – 573.17*	554.05	555.33 (5.83)
	Bio17	*Prec. of Driest Quarter (mm)*		*98.02 – 105.00*	99.83	100.04 (1.46)
	Bio18	*Prec. of Warmest Quarter (mm)*		*105.00 – 112.01*	106.96	107.19 (1.36)
	Bio19	*Prec. of Coldest Quarter (mm)*		*548.05 – 573.17*	554.05	555.33 (5.83)
microclimate	*lstsummer*	*Summer Land Surface Temperature (ºC)*		*27.63 – 40.64*	32.92	32.84 (2.13)
	lstwinter	*Winter Land Surface Temperature (ºC)*		*6.80 – 13.61*	10.38	10.35 (1.01)
	ndvisummer	*Summer Normalized Difference Vegetation Index*	More information on data acquisition in the supplementary material 2.	*0.10 - 1.0*	0.82	0.78 (0.16)
	ndviwinter	*Winter Normalized Difference Vegetation Index*		*0.07 - 1.0*	0.61	0.60 (0.14)

Results

Sampled trees

We georeferenced 7357 large trees in Lousada (Figure 2.1). From these, 2807 (38.2%) trees were characterized at the TreMs diversity level, its management and the surrounding environment. It was possible to identify 56 species/genus of trees. From those, ten tree species make up 85.7% of the whole subset. The most common species were *Platanus* sp. and *Quercus robur*, with 746 and 675 trees. Followed by *Populus nigra*, *Quercus suber* and *Cupressus* sp. with 196, 169 and 148 trees, respectively. The tree species with fewer specimens was *Eucalyptus globulus* with 81 individuals (Figure 2.2).

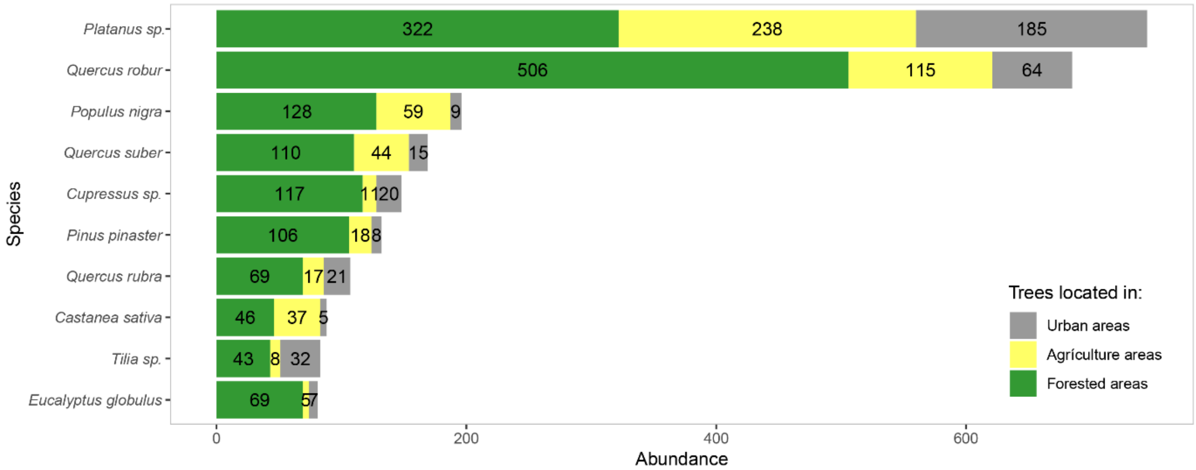


Figure 2.2- Abundance of the ten most common species inventoried at the Municipality of Lousada according to the different land uses where they are located.

Tree species with the highest median value of TreMs diversity was *Tilia* sp. and *Q. robur*, followed by *Cupressus* sp. and *Castanea sativa* (Figure 2.3). *Q. robur* is the species with the absolute highest value of TreMs diversity with the maximum value of 27.

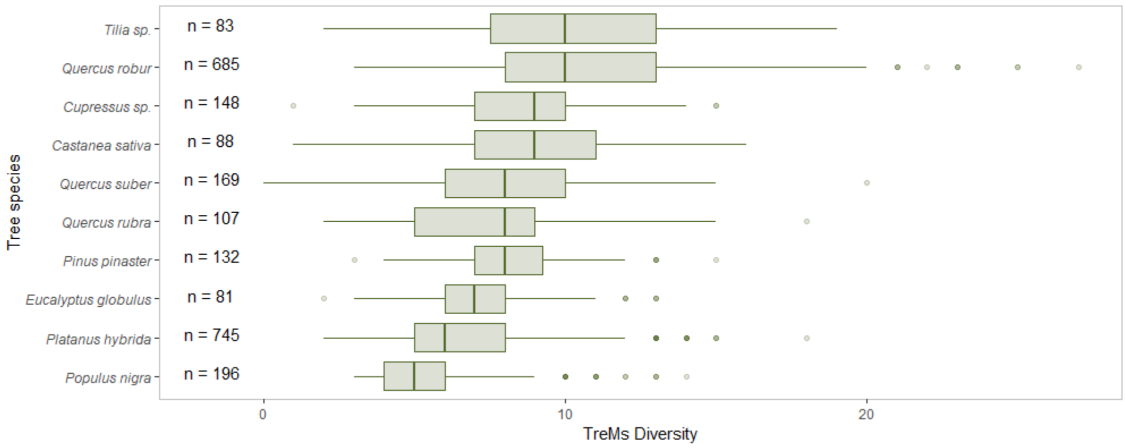


Figure 2.3 - Boxplot of the distribution of the TreMs diversity for each of the species with more than 80 trees in the sampled group.

TreMs Composition

The NMDS ordination plot (Figure 2.4) shows the location of each TreMs category based on the TreMs category richness of each sampled tree. With a final model stress of 19.8%, axis 1 explained 56.3% of the variation in TreMs category richness, and axis 2 explained 24.0% in a total of 80.3%. The first axis of the ordination represents a land-use artificialization gradient from forest to urban areas. Here, species origin, weather is native or non-native (*Or*, r=-0.42, p < 0.001), land use where tree is growing (*LU.tg*, r=-0.25, p<0.001) macroclimatic variables such has annual range of temperature (*Bio7*, r=0.21, p<0.001) and precipitation of the warmest quarter (Bio18, r=-0.19, p<0.001) show the highest correlation with the axis. Several variables showed significant correlations with axis 1, although some of them showed a higher correlation with axis 2, as pruning frequency (*Pr.freq*, r=0.27, p<0.001) and area of urban land surface (*LU.urb*, r=0.21,p<0.001) (Table 2.2).

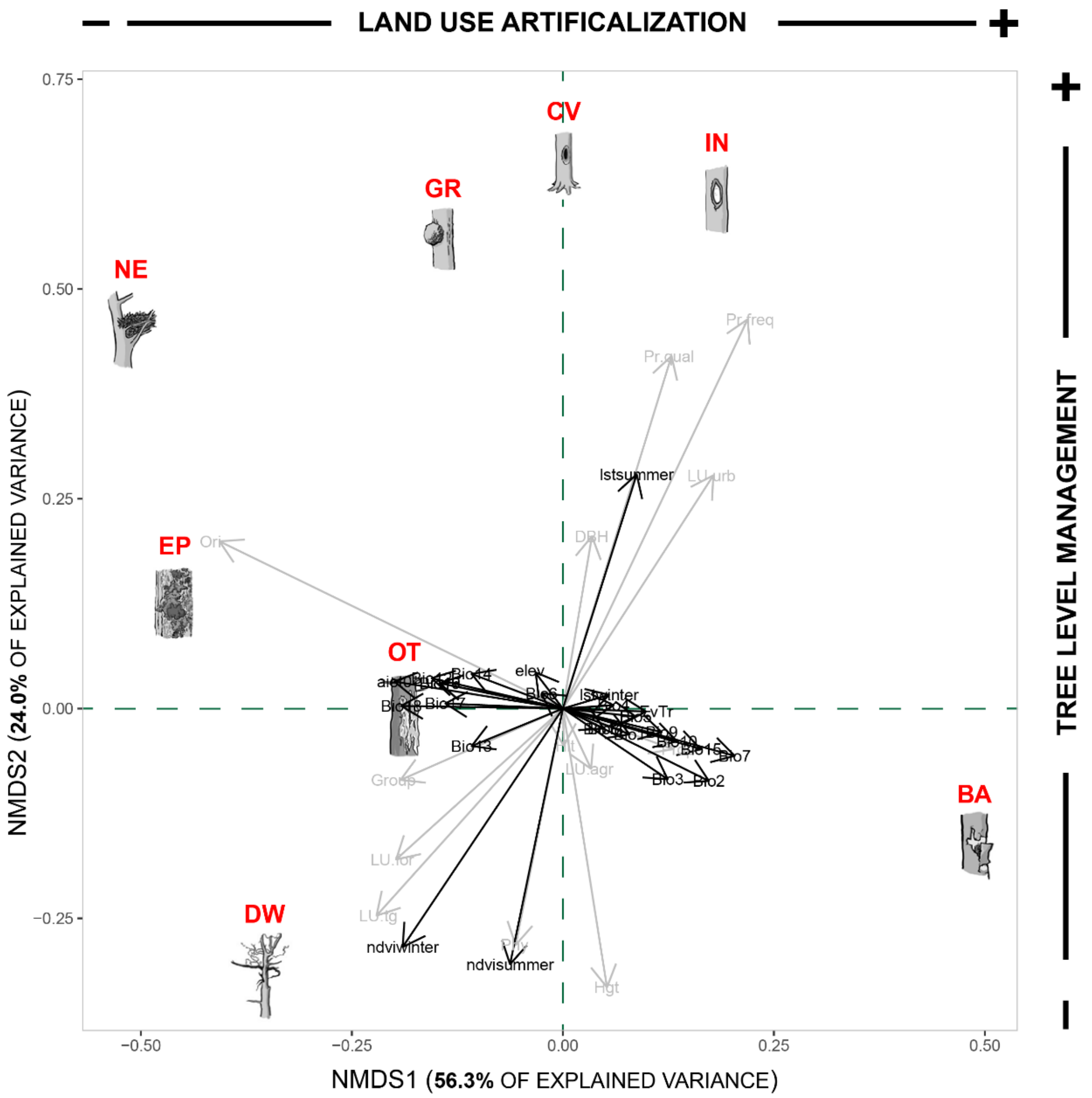
The second axis of the ordination represents a gradient of tree level management intensity, ranging from no management to high intensity management. Pruning frequency (*Pr.freq*, r=0.43, p<0.001) and quality (*Pr.qual*, r=0.41, p<0.001) are the variables with higher correlation with axis 2, followed by tree height (*Hgt*, r=-0.33, p<0.001) and microclimatic variables such as the summer NDVI (*ndvisummer*, r=-0.30, p<0.001), summer LST (*lstsummer*, r=0.27, p<0.001) and winter NDVI (*ndviwinter*, r=-0.26, p<0.001).

TreMs categories were highly correlated with the ordination plot axis, with bark (*BA*, r=0.74, p<0.001), deadwood (*DW*, r=-0.61, p <0.001) and epiphytes (*EP*, r=-0.54, p<0.001) being highly correlated with axis 1 and injuries (*IN*, r=0.75, p<0.001), cavities (*CV*, r=0.48, p<0.001) and growth forms (*GR*, r=0.37, p<0.001) highly correlated with axis 2 (Figure 2.4).

Based on axis 1, in less artificialized areas, i.e., forests, TreMs that are naturally created are more common (such as deadwood, nests and epiphyte) whilst as artificialization increases, TreMs resulting from human action are more common, such as wounds. Species origin is highly correlated with axis 1, with native species more common in less artificialized areas. Exotic species are less used by other organisms to create TreMs and so, the most common TreMs in exotic species are those created by the tree itself, resulting from its own defences against outside pathogens (bark characteristics) and those created by humans, such as wounds, being these the ones found on the positive extreme of the axis 1.

Axis 2 showed an increased intensity in tree management, with deadwood present in low managed areas and wounds resulting from pruning in the other extreme. Bad pruning consequently increases cavities and growth forms from tree infections, resulting in a high correlation of this TreMs with this axis.

More complex compositions of TreMs are found in low artificialized areas with average quality in management.



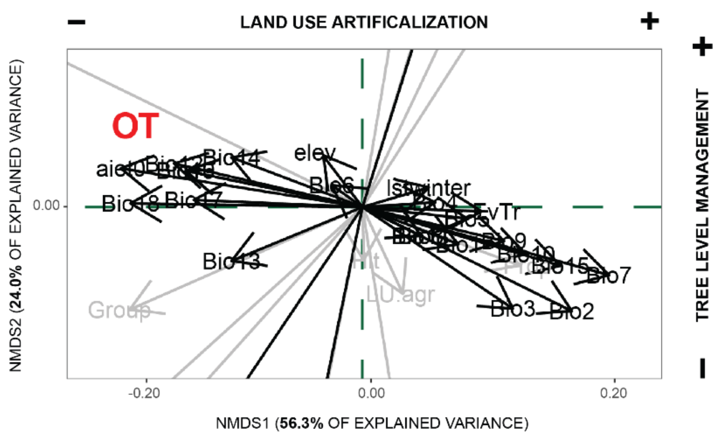


Figure 2.4 - Ordination plot of the non-metric multidimensional scaling (NMDS) performed on a matrix of TreMs categories diversity per sampled large tree (CV – Cavities; IN – Injuries, BA – Bark; DW – Crown Dead Wood; EP – Epiphytes; NE – Nests, GR – Growth forms; OT – Others). A focus on the origin point of the ordination plot is available so that a detailed analysis can be executed.

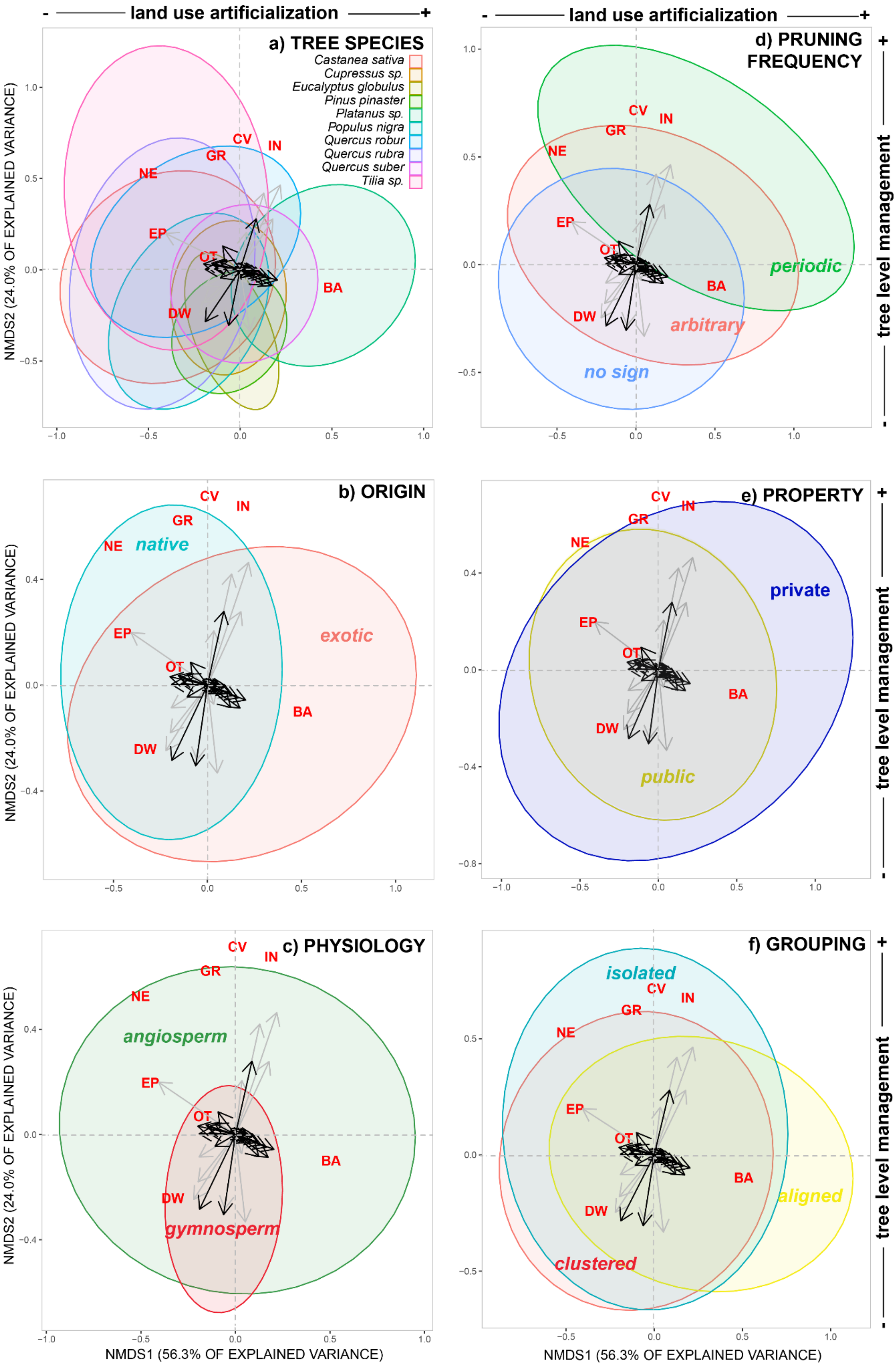


Figure 2.5 - Simplified ordination plot data grouping according to tree traits: a) tree species, b) tree origin, c) tree physiology, d) pruning frequency, e) property and f) grouping.

Table 2.2 - Spearman correlations coefficients between TreMs variables, environmental variables and the ordination plot axis 1 and 2.

Variables category	**Variable code**	**Variable name**	NMDS main axis NMDS1	NMDS2
TreMs Categories	CV	Cavities	-0.06**	0.48***
	IN	Injuries and Wounds	0.05*	0.75***
	BA	Bark	0.74***	-0.08***
	DE	Deadwood	-0.61***	-0.27***
	GR	Deformation/ Growth form	-0.09***	0.37***
	EP	Epiphytes	-0.54***	0.35***
	NE	Nests	-0.09***	0.12***
	OT	Others	-0.07**	0.06**
TREE TRAITS	PBH	Perimeter at Breast Height	0.05*	0.20***
	Hgt	Height	0.07***	-0.33***
	Hlt	Health	0.00	-0.04*
	Or	Origin	-0.42***	0.16***
	Ph	Physiology	-0.07***	-0.28***
TREE MANAGEMENT	Group	Grouping	-0.20***	-0.07***
	Prop	Property	0.13***	-0.04
	Pr.freq	Pruning frequency	0.27***	0.43***
	Pr.qual	*Types of wrong cuts from pruning*	0.17***	0.41***
	LU.urb	*The surrounding area of urban land use (m2)*	0.21***	0.25***
	LU.agr	*The surrounding area of agriculture land use (m2)*	0.04*	-0.07***
	LU.for	*The surrounding area of forest land use (m2)*	-0.22***	-0.15***
	LU.tg	*Land use where the tree is growing*	-0.25***	-0.21***
MACROCLIM	Arid	*Global Aridity Index*	-0.20***	0.02
	EvTr	*Global Reference Evapo-Transpiration (mm/day)*	0.10***	0.00
	elev	*Elevation*	-0.04	0.04
	Bio1	*Annual Mean Temperature (ºC)*	0.08***	-0.02
	Bio2	*Mean Diurnal Range (ºC)*	0.18***	-0.07***
	Bio3	*Isothermality (ºC)*	0.13***	-0.07***
	Bio4	*Temp. Seasonality (ºC)*	0.06***	0.00
	Bio5	*Max Temp. of Warmest Month (ºC)*	0.09***	-0.01
	Bio6	*Min Temp. of Coldest Month (ºC)*	-0.03	0.01
	Bio7	*Temp. Annual Range (ºC)*	0.21***	-0.04*
	Bio8	*Mean Temp. of Wettest Quarter (ºC)*	0.05**	-0.02
	Bio9	*Mean Temp. of Driest Quarter (ºC)*	0.12***	-0.02
	Bio10	*Mean Temp. of Warmest Quarter (ºC)*	0.14***	-0.03
	Bio11	*Mean Temp.of Coldest Quarter (ºC)*	0.05***	-0.02
	Bio12	*Annual Precipitation (mm)*	-0.16***	0.03
	Bio13	*Prec.of Wettest Month (mm)*	-0.11***	-0.03
	Bio14	*Prec. of Driest Month (mm)*	-0.11***	0.03
	Bio15	*Prec. Seasonality (mm)*	0.17***	-0.04
	Bio16	*Prec of Wettest Quarter (mm)*	-0.15***	0.02
	Bio17	*Prec. of Driest Quarter (mm)*	-0.14***	0.00
	Bio18	*Prec. of Warmest Quarter (mm)*	-0.19***	0.00
	Bio19	*Prec. of Coldest Quarter (mm)*	-0.15***	0.02
MICROCLIM	lstsummer	*Summer Land Surface Temperature (ºC)*	0.11***	0.27***
	lstwinter	*Winter Land Surface Temperature (ºC)*	0.06**	0.01
	ndvisummer	*Summer Normalized Difference Vegetation Index*	-0.08***	-0.30***
	ndviwinter	*Winter Normalized Difference Vegetation Index*	-0.23***	-0.26***

TreMs Diversity

Model 1 showed that TreMs diversity varies with tree species/genus, with pedunculated oak (*Q. robur*) having the highest TreMs diversity among the ten most common tree species (Table 2.3). Every species showed significant differences regarding *Q. robur,* with *Platanus sp.* and *E. globulus* showing the highest difference ($r=-0.42$, $p<0.001$ and $r=-0.39$, $p<0.001$, respectively). Tree PBH was also positive and significantly correlated with TreMs diversity. Tree height had the opposite relation, i.e., although significantly, it was negatively correlated with TreMs. Regarding tree management, the trees grouping also showed differences regarding its levels. When comparing with isolated trees, clustered trees have higher TreMs diversity than isolated ones ($r=0.09$, $p<0.05$) and aligned ones have less ($r=-0.09$, $p<0.05$). Trees in private areas also showed higher diversity than public ones ($r=0.156$, $p<0.001$). Pruning frequency also had a positive and significant association with TreMs diversity. Regarding macro and microclimate variable, high annual range of temperatures showed a positive relation with higher TreMs diversity ($r=0.21$, $p<0.01$) and smaller values of evapotranspiration too ($r=-0.05$, $p<0.05$).

Model 2 showed that tree origin and physiology have significant correlations between TreMs diversity and native trees ($r=0.24$, $p<0.001$) or gymnosperms ($r=0.08$, $p<0.01$). Regarding the remaining variables of tree traits, tree management and macroclimate responded in the same way as in Model 1. Regarding microclimate, we found a positive and significative relation between TreMs and summer land surface temperature, meaning that warmer areas had higher diversity of TreMs ($r=0.014$, $p<0.05$) (Table 2.3).

Model 3 showed that a simplified model with only tree species, height and PBH has similar validation results when compared to the explanatory models 1 and 2 (with similar deviance and correlation test results) indicating that these variables can be exclusively used to explain the TreMs diversity of large trees.

Table 2.3 - Generalized linear models results regarding the variables that were retained from the NMDS. Model 1 was based on taxonomical differences regarding tree species and model 2 regarding tree origin and physiology. Model 3 is a simplified predictive model with only 3 variables. "ref" indicates the reference level within the category that the model coefficient (coef) was compared to, se indicates the Standard Error and p the p-value.

			MODEL 1			MODEL 2			MODEL 3		
			coef	se	p	coef	se	p	coef	se	p
		Intersection	2.967	(1.950)		4.160	(1.911)	*	1.973	(0.047)	***
TREE TRAITS	*Spp.*	*Quercus robur*	ref						Ref		
		Pinus pinaster	-0.118	(0.042)	**				-0.157	(0.042)	***
		Tilia sp.	-0.120	(0.047)	*				-0.165	(0.047)	***
		Cupressus sp.	-0.127	(0.038)	***				-0.155	(0.036)	***
		Castanea sativa	-0.133	(0.045)	**				-0.186	(0.045)	***
		Quercus suber	-0.219	(0.038)	***				-0.307	(0.037)	***
		Quercus rubra	-0.236	(0.047)	***				-0.328	(0.047)	***
		Populus nigra	-0.389	(0.044)	***				-0.511	(0.042)	***
		Eucalyptus globulus	-0.390	(0.055)	***				-0.415	(0.055)	***
		Platanus sp.	-0.421	(0.029)	***				-0.469	(0.023)	***
	Or	*Native*				0.243	(0.023)	***			
		Exotic				Ref					
	Ph	*Gymnosperm*				0.075	(0.028)	**			
		Angiosperm				Ref					
	PBH		0.007	(0.001)	***	0.007	(0.001)	***	0.008	(0.001)	***
	Hgt		-0.005	(0.002)	**	-0.008	(0.002)	***	-0.009	(0.001)	***
TREE MANAGEMENT	*Group*	*Isolated*	Ref								
		Clustered	0.085	(0.036)	*	0.126	(0.036)	***			
		Aligned	-0.084	(0.040)	*	-0.095	(0.040)	*			
	Prop	*Private*	Ref								
		Public	-0.156	(0.024)	***	-0.171	(0.025)	***			
	Pr.freq	*No signal*	Ref								
		Arbitrary	0.140	(0.021)	***	0.158	(0.020)	***			
		Periodic	0.341	(0.047)	***	0.340	(0.046)	***			
	LU.urb					0.000	(0.000)				
	LU.for		0.000	(0.000)	*	0.000	(0.000)	***			
MACROCLIM	*Bio7*		0.211	(0.077)	**	0.222	(0.078)	**			
	EvTr		-0.005	(0.002)	*	-0.007	(0.002)	***			
MICROCLIM	*ndvisummer*					-0.101	(0.070)				
	lstsummer					0.014	(0.006)	*			
VALIDATION	DEVIANCE		0.445			0.394			0.395		
	PEARSON CORRELATION TEST		0.633***			0.596***			0.607***		

Discussion

In this work we show that for each tree, the available TreMs are the result of a combination of the tree species, its specific traits, mostly height and PBH, the historical management that was applied to it and the micro and macroclimatic conditions of its surrounding environment. TreMs diversity is highly related with tree species traits although tree management and surrounding climate have a role in the formation of some structures. This is highlighted when analysing TreMs compositions since these vary along both a land use artificialization and a tree management gradient. In other words, TreMs composition changes with the gradient from forests to urban land-use and in relation with tree level management, from low to high frequency of interventions. Further, a simplified GLM that only accounts for species traits has similar results with those that account for more variables, although not reaching its full potential. Here we will discuss these findings and the importance of large trees and associated TreMs as bioindicators of saproxylic biodiversity.

Drivers of TreMs Composition and Diversity

TREE TRAITS

Specific tree traits are extremely important to understand their capacity to support and originate certain TreMs. With this work we found that TreMs diversity varies within taxa, and *Q. robur* is the tree species with highest TreMs diversity followed by *C. sativa* and *Tilia* sp.. On the other hand, *E. globulus*, *Platanus* sp. and *P. nigra* are the large trees that show the poorest TreMs diversity. Mitchell et al (2019) found that British oaks (*Q. robur/petraea*) are the most important tree species in the UK in terms of nature conservation, supporting 2300 species of animals, plants, bryophytes, fungi, mostly associated with its bark, deadwood, leaves, limbs and branches can help understand these results. In their work, no other species can support such a great amount of the oak-associated species (maximum reached by *Fraxinus excelsior*, with 28%) since there are physiological differences between different species that do not allow that ecological relationships can be established between the organisms and their host trees, leading, in some cases in lacks of formation of TreMs such has cavities, growth-forms and epiphytes.

Trees with larger PBH contained higher TreMs diversity, since, in our study, high PBH is linked to the formation of cavities, injuries and growth forms. Trees with high PBH have higher probabilities of going through microhabitat-creation events resulting from their longer lifespan (e.g., diseases, parasite attacks, climatic events, and harvesting damage (Vuidot et al. 2011; Regnery et al. 2013) or even periodic pruning leading to the formation of more complex TreMs compositions. For example, trees with high PBH are in general preferred by large saproxylophagous organisms such as the Great Capricorn beetle (*Cerambyx cerdo*) that creates large bore holes in the trunk (TreM CV52). This species normally prefers larger oak tree trunks however it is not a limiting feature; it searches for trees that have suitable wood for the development of its larvae, normally sun exposed and with some extent of decay (mostly found in large and old tree specimens) (Platek et al. 2019). Stokland and colleagues (2012) also reviewed factors that cause tree trunk dimension effects on saproxylic species preferences and one of the highlighted characteristics was that as a tree grows, normally its bark also gets thicker, mainly at the base of the tree. Bark thickness in living trees is related to highly abundance of TreMs and its usage (Michel et al. 2011) and as thick as the bark gets, it increases its performance as temperature insulator, and pathogen protection. This insulator effect is increased by the smaller surface area to volume ratio found in large trees (Stokland et al. 2012).The correlation between high PBH and high TreMs diversity has been extensively corroborated by several different studies in a variety of forest ecosystems (e.g., Winter and Möller 2008; Michel and Winter 2009; Ragón et al. 2010; Vuidot et al. 2011; Larrieu and Cabanettes 2012; Regnery et al. 2013; Bütler et al. 2013; Bouget et al. 2014; Johann and Schaich 2016; Abdullah et al. 2017; Großmann et al. 2018; Kraus et al. 2018; Asbeck et al. 2019).

On the other side, tree height is negatively correlated with TreMs diversity. Further from the fact that in our study, taller-slim trees are mostly individuals of *E. globulus*, *P. pinaster* and *P. nigra* that are found in low managed forested areas, and that when they become larger are cut because of their timber value, other species that can reach large perimeter have less TreMs when they reach the pick of their height, where after normally tend to become lower and with larger trunks, as a result of natural decay. Since tall and slim species normally do not reach old-growth stages in anthropogenic landscapes, it is expected that they do not accumulate great amounts of TreMs related to stochastic events such has cavities and wounds. Furthermore, due to their small life spans, their colonisation e.g. by epiphytic organisms can be compromised. In our case, height seems to be more correlated with bark and deadwood TreMs resulting from these species natural features.

Another important trait is the phylogenetic origin of the tree. In our work we found that, although both native and exotic trees host diverse TreMs compositions, native trees are more prone to be colonised by a higher diversity of organisms, hosting a wide variety of nests, epiphytes, growth forms and cavities. Both the works of Meijer and colleagues (2015) and Southwood and colleagues (1982) showed that species diversity of phytophagous organisms (i.e. those that feed on the trees organs) is higher for native plants than for exotic ones. This can be explained by the result of millions of years of co-evolution with other organisms that evolved with the tree species and are able to create TreMs for their own use (Stokland et al. 2012).

Additionally, according to our results, gymnosperms have high TreMs diversity than angiosperms (contrasting with analysis e.g. from Asbeck et al. (2019) or Großmann et al. (2018), both indicating that in European forests, broadleaved trees (angiosperms) have potential overall higher TreMs diversity that conifers (gymnosperms)), although their composition is more homogeneous. An explanation for this contrast result relates with the different levels of analyses of TreMs. The composition analysis shows that while angiosperms have TreMs in eight categories, gymnosperms have mostly TreMs connected to only three categories, DW - dead branches from natural death, BA - bark structures and the occurrence of OT - resin/sap runs, with high diversity within these three TreMs categories.

MANAGEMENT CHARACTERISTICS

TreMs compositions vary significantly regarding the type of management applied to the trees. Land use leads to different tree species richness and the applied management since, according to the land use, different social and economic goals need to be fulfilled by the present trees.

In natural forested areas with high tree species richness, the TreMs that trees host are mainly a result of the natural variability of structural traits that different tree species have, that are naturally created during the tree growth (e.g., different woody material, bark types, branches and limbs shapes within species) and that influence the colonisation capacity of other organisms that use the tree as support (EP) or to create nests (NE). Additionally, the low management applied to these trees allows the formation of TreMs that are normally difficult to find in other land uses such as deadwood in the trees canopy (DW). Contrarily, highly artificialized areas have poor tree species richness and consequently homogenised TreMs compositions. Pruning frequency and quality has a high significant correlation, in both axes of the ordination plot being linked with both major variations in land use and tree level management. Its increase of frequency leads to changes in the TreMs compositions since it brings an increase in diversity of injuries occurring in the trees. In the Lousada's case, urban areas TreMs compositions are highly homogenised as a cumulative effect of low tree species richness, being mostly dominated by *Platanus sp.* (a species with low TreMs diversity values) but also of a homogenised pruning frequency and quality, leading to TreMs compositions dominated by injuries (IN), cavities (CV) and bark structures (BA) and a complete lack of deadwood in the trees canopies (DW). Scientific literature linked the formation of TreMs by human in two distinct perspectives. The first, linked to cultural value of the trees, applied management is seen as a promoter of the tree death, being identified as a threat for the survival of large trees (Lopes et al. 2019). On the other side, ecologist have identified traditional pruning such has coppicing and pollarding as a mean to develop larger trees and richer TreMs compositions in them, and ultimately promote a cultural and economic link between people and the trees. Furthermore, it is suggested as a mean to fill habitats gaps in artificialized areas and improve the TreMs diversity of normally poor areas (Sebek et al. 2013; Le Roux et al. 2014; Ramilo et al. 2017).

Clustered and isolated trees have higher TreMs diversity than aligned ones, hosting more diversified TreMs compositions. In our case, aligned trees appear to be linked to agriculture areas and isolated/clustered trees can be found in two or more land use types. This suggests that the TreMs compositions that trees have is mostly an outcome of the land use type where they are settled, although their disposition might affect the applied management and colonisation by organisms. For example, isolated trees can be found in the whole spectrum of land uses, as any species and with or without pruning. The result is a group of trees that can have all the types of TreMs, and the composition is normally rich and dependent on other variables.

Moreover, privately-owned trees have higher TreMs diversity than public ones. This is probably a result of the low area managed by public entities (only 27% of the sampled trees) and that normally need to fulfil social goals, beingly highly managed to avoid public safety issues. Regarding TreMs composition is possible to see that private trees have richer compositions of TreMs, including almost all TreMs categories in them. Johann and Schaich (2016) had the same results for German forests, where small-scale private areas had twice as high density of TreMs than public managed ones. If we see it in a large-scale perspective, the cumulative effect of different management practices by small owners can lead to a higher variety of TreMs, in contrast with public management that might only vary based on the knowhow of the technicians that manage the trees or the social goals that the trees need to fulfil.

MACRO/MICROCLIMATE CHARACTERISTICS

Our results, although showing significant correlations, show that variations in microclimate variables at our small landscape scale are mostly linked to land use artificialization and consequent management.

Our data shows that macroclimatic variables are mainly linked to bark structure (BA), its colonisation by epiphytic organisms (EP) or its failure to protect the tree against pathogens leading to the formation of sap and resin runs (OT). Climatic factors have an impact in the defence mechanisms of trees and its colonisation by epiphytic organisms that may also help with tree decomposition, such has fungi fruiting bodies. Regarding microclimate variables such as *ndvi* and *lst*, both are highly correlated with axis 1 of the ordination plot. Higher *lst* values are found in urbanized areas and led to TreMs compositions dominated by tree injuries and consequent cavities. Regarding *ndvi*, higher values are found in forested areas and are highly linked to TreMs found in these areas like deadwood.

It has been highlighted, e.g. by Stokland et al. (2012) that abiotic environment has a high influence on the conditions that allow the use of TreMs by organisms, being the most important temperature, moisture and oxygen pressure. Furthermore, according to Hatfield and Prueger (2015) temperature surrounding the plants influences its growth and reproduction, with each species having a specific temperature range of optimum development. Temperature variations and drought are also linked to tree mortality (Mcdowell et al. 2008; Alliere et al. 2019) (especially in larger trees (Bennett et al. 2015)) and increased pathogen colonisation (Roy et al. 2004).

PREDICTING TREMS DIVERSITY

Our results show that a simplified combination of species traits (species + PBH + height) is able to explain the diversity of TreMs in trees almost as much as the combination of tree traits, management and macro/microclimate variables (Model 1 and 2, Table 2.3), showing that these three variables are the most important to calculate the diversity of TreMs in large trees. These results help to acknowledge the value of large trees as keystone elements. Although, our composition analysis shows that, throughout the landscape, TreMs compositions in trees vary, even if the diversity (total number of different TreMs) doesn't. Accounting for it, a simplified analysis of the importance of large trees for the conservation of saproxylic biodiversity can be calculated, although only assessing the diversity of elements. We need to have in mind that in a diversified landscape, the TreMs compositions are different between, i.e. forests and urban areas and that, for now, this simplified model cannot be used to prove that TreMs diversity is a bioindicator of the saproxylic biodiversity of complex and heterogenic systems.

TREE PRESERVATION IN LOUSADA

Lousada's major land use changes are linked to increased urban areas in the place of agricultural and forested areas (Abrantes et al. 2018). Unpublished data from the municipality shows that around 3% of large trees are cut yearly in Lousada, with increased urbanization being the main known cause of destruction (40% of the cut trees), followed by agriculture intensification (17%), personal use (12%) and public safety (11%) with natural death only accounting for 5% of the cutting causes. When extrapolated for the future, in 20 years we can expect a disappearance of more than 45% of the identified large trees.

We suggest that trees (especially oaks) with large diameter should be increasingly valued and protected at the municipality scale. To achieve long term goals, a social valorisation of these trees is necessary according to the different land use types and its influence on the tree species distributions and economical mechanisms to promote better conservation practices by private owners might also be one of the strategies, although both counterparts need to be aligned and work together to achieve greater goals, especially in what comes to land use change.

Lousada's large trees can be found mostly in forests and agricultural areas. In forest areas tree species are dominated by *E. globulus* (61% of the municipal forested area), *P. pinaster*, *P. nigra*, with distributed remnants patches of *Q. robur*, cork oaks (*Q. suber*), American oaks (*Q. rubra*) and invasive species such has *Acacia* spp. or *Robinia pseudoacacia*. The great diversity of trees in this land-use leads to diversified TreMs compositions than in other areas, as a result of a higher abundance of TreMs per area (since more trees are available) but also because, as explained before, the variability of taxa leads to variability of habitats. Regarding agricultural areas, large trees are mostly used by citizens to collect fruits such has walnuts (*Juglans regia*) and chestnuts (*C. sativa*), but also to support wine yards in traditional agricultural methods called "Vinha do Enforcado" using mostly *Platanus sp.*, European hakeberries (*Celtis australis*) and *Q. robur*. "Vinha do Enforcado" trees are actively pruned to control the tree growth leading to the formation of a great abundance and diversity of TreMs in these trees, even if the species is exotic and not reaching high PBH. An assessment of the TreMs diversity of these managed trees should be elaborated since they link socio-economical management with ecological valorisation of trees and perhaps an ongoing valorisation of native trees that are able to fulfil this demanded social goals can be used as a integrative measure to improve saproxylic biodiversity of these areas.

In urban centres the most common species are *Platanus sp.* and are managed in a homogeneous way to fulfil social goals. An increased richness of tree species that are able to reach high PBH should be one of the first goals of the municipality, and to achieve fast results, the large urban trees that are not *Platanus sp*. should be preserved at all cost. Furthermore, promoting different management mechanism can lead to an ecological valorisation of the current large trees, where even creating, in some cases, artificial habitats can help (Le Roux et al. 2014). Residential areas and old farms with big gardens, that are part of the rural landscape, are mostly dominated by large old trees such has *Q. robur*, *Tilia sp.*, *Cupressus sp*. and other botanic rarities that lead to a variation of TreMs compositions that is integrated in the surrounding agricultural or forested environment. These farms allowed the preservation of trees during long periods of time and its possible to find in them some particular specimens of outstanding dimensions and TreMs diversity. The ongoing work with private landowners, with small or large areas, should continue and be improved since it has a high potential of TreMs preservation on a long term.

POTENTIAL FOR TREE PRESERVATION IN PORTUGAL

In a broader angle and to change the future perspectives regarding tree preservation in Portugal we propose:

1) A change of concept to identify and protect trees, allowing a larger valorisation of large trees in Portugal based also on their ecological value, promoting a broader conservation of these specimens;
2) A TreMs assessment of the already identified monumental trees, allowing to understand the relation between their already valued social importance and their ecological role;
3) A continued TreMs assessment of large trees of more species and habitats, helping to improve the predicting models;
4) The creation of a national scale for large tree identification and characterization database (possibly creating a citizen-science project like the one in the UK) with continuous monitoring;
5) The promotion of social valorisation of these trees using them as touristic attractions or as live laboratories to educate regarding nature conservation and natural heritage;
6) The creation of economic valorisation of these trees as a mean to protect them from being cut without valid reasons.

Conclusions

The large tree composition and diversity of TreMs is multifactor, i.e., it depends on the combination of tree traits and environmental variables that are important for the TreMs assemblies that trees host. Tree traits such has its species and its dimensions (PBH and Height) are vital to understand the potential of trees to host saproxylic organisms, but specifically where the TreMs occur and how to manage them. Furthermore, human management as a complementary importance in the formation of TreMs, leading to different compositions of these elements on trees. The promotion of large trees preservation in southwestern Europe is dependent of social-economic factors, that nowadays simply result in a decreased valorisation of the ecological value that large trees have. TreMs should be used as tool to identify trees with potential for conservation, since it allows to assess tree traits related with high ecological potential value. Our study also suggests that native trees (especially oaks), privately owned or grouped, with a large and not that high trunk should be increasingly valued and protected in Lousada and potentially at a regional or national scale. Pruning has the potential of creation of TreMs and consequently improve the saproxylic community, although, since mostly done in urban exotic species, leads to homogenised TreMs assemblages in these areas. A more diversified tree species assemblages in urban areas might lead to a change in TreMs compositions and consequently to an increased ecological potential. Moreover, since TreMs formation is influenced by climatic variables and are deadwood ecological niches, the increased alterations in climate might lead to increases in the creation of these structures, eventually leading to the death of trees. It is then expected that future climate change will have a role in TreMs composition throughout the landscape, influencing mostly those related to the death of trees or pathogens colonisation. National and local tree preservation is needed in Portugal and for that, a social, economic and ecological valorisation is necessary, accompanied by more strict regulation regarding large tree management. To acknowledge the ecological potential of large trees, simplified predictions of its TreMs diversity can be used if based on the tree species, its trunk perimeter and total height, although having in mind that, according to the land use where the tree is growing, a particular assembly of TreMs are more probably available than others. Since these variables are measured at large scales in forest inventories, the calculation of the TreMs diversity in a broader spatial scale can be executed based on already collected data.

Acknowledgements

We would like to appreciate all the help from the volunteers and colleagues that participated in field work (Mathilde Claes, Gonçalo Abreu; Marta Pinto; Eduarda Barbosa; Cláudia Silva; João Pereira; Mariana Cardoso; Pedro Freitas; Ivo Rosa; Cláudia Mendes; Mariana Carqueijeiro; Inês Ferreira, Francisco Leal; Joana Rocha, Joana Pereira; André Couto, Pedro Sá, Rafael Marques, Diego Alves, Daniela Barbosa, Luis Cunha and Ernesto Gonçalves), the Integrated+ Project team for allowing us to use some images from the Catalogue of Microhabitats in our work and for all the help understanding it (specially Andreas Schuck); and finally but not the least, all Lousada citizens who opened the doors of their houses and properties and allowed us to characterize their trees.

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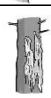
Supplementary Material 1

Tree related microhabitats (TreMs) measured accordingly to the Catalogue of microhabitats (Kraus et al. 2016) in the Lousada municipality, Portugal.

CATEGORY OF TREMS	SUBCATEGORY OF TREMS	TREM	Code
CAVITIES (CV) 	WOODPECKER CAVITIES	ø = 4cm	CV11
		ø = 5-6 cm	CV12
		ø >10cm	CV13
		ø ≥ 10cm (feeding hole)	CV14
		Woodpecker "flute" / cavity string	CV15
	TRUNK AND MOULD CAVITIES	ø ≥ 10cm (ground contact)	CV21
		ø ≥ 30cm (ground contact)	CV22
		ø ≥ 10cm	CV23
		ø ≥ 30cm	CV24
		ø ≥ 30cm/ semi-open	CV25
		ø ≥ 30cm/ open top	CV26
	BRANCH HOLES	ø ≥ 5 cm	CV31
		ø ≥ 10cm	CV32
		Hollow branch, ø ≥ 10cm	CV33
	DENDROTELMS AND WATER-FILLER HOLES	ø ≥ 3 cm/ trunk base	CV41
		ø ≥ 15cm/ trunk base	CV42
		ø ≥ 5cm/ crown	CV43
		ø ≥ 15cm/ crown	CV44
	INSECT GALLERIES AND BORE HOLES	Gallery with single small bore holes	CV51
		Large bore hole ø ≥ 2 cm	CV52
INJURIES AND WOUNDS (IN) 	BARK LOSS/ EXPOSED SAPWOOD	Bark loss 25- 600 cm^2, decay stage < 3	IN11
		Bark loss > 600 cm^2, decay stage < 3	IN12
		Bark loss 25- 600 cm^2, Decay stage =3	IN13
		Bark loss > 600 cm^2, Decay stage =3	IN14
	EXPOSED HEARTHWOOD/ TRUNK AND CROWN BREAKAGE	Broken trunk, ø ≥ 20 cm at the broken end	IN21
		Broken tree crown / fork; Exposed wood ≥ 300 cm^2	IN22
		Broken limb, ø ≥ 20 cm at the broken end	IN23
		Splintered stem, ø ≥ 20 cm at the broken end	IN24
	CRACKS AND SCARS	Length ≥ 30 cm; width > 1 cm; depth > 10 cm	IN31
		Length ≥ 100 cm; width > 1 cm; depth > 10 cm	IN32
		Lightning scar	IN33
		Fire scar, ≥ 600 cm^2	IN34
BARK (BA) 	BARK POCKETS	Bark shelter, Width > 1 cm; depth > 10 cm; height > 10 cm	BA11
		Bark pocket, Width > 1 cm; depth > 10 cm; height > 10 cm	BA12
	BARK STRUCTURE	Coarse bark	BA21
DEADWOOD (DW) 	DEAD BRANCHES AND LIMBS / CROWN DEADWOOD	ø 10 - 20 cm, ≥ 50 cm; sun exposed	DW11
		ø > 20 cm, ≥ 50 cm; sun exposed	DW12
		ø 10 - 20 cm, ≥ 50 cm; not sun exposed	DW13
		ø > 20 cm, ≥ 50 cm; not sun exposed	DW14
		Dead top ø ≥ 10 cm	DW15
DEFORMATIONS / GROWTH FORMS (GR) 	ROOT BUTTRESS CAVITIES	ø ≥ 5 cm	GR11
		ø ≥ 10 cm	GR12
		Trunk cleavage; length ≥ 30 cm	GR13
	WITCHES BROOM	Witches broom, ø > 50 cm	GR21
		Water sprout	GR22
	CANKERS AND BURRS	Cancerous growth, ø > 20 cm	GR31
		Decayed canker, ø > 20 cm	GR32
EPIPHYTES (EP) 	FRUITING BODIES FUNGI	Annual polypores, ø > 5 cm	EP11
		Perennial polypores, ø > 10 cm	EP12
		Pulpy agaric, ø > 5 cm	EP13
		Large ascomycetes, ø > 5 cm	EP14
	MYXOMICETES	Myxomycetes, ø > 5 cm	EP21
	EPIPHYTIC CRYPTO- AND PHANEROGAMS	Epiphytic bryophytes coverage > 25 %	EP31
		Epiphytic foliose and fruticose lichens, coverage > 25 %	EP32
		Lianas, coverage > 25 %	EP33
		Epiphytic ferns, > 5 fronds	EP34
		Mistletoe	EP35
NESTS (NE) 	VERTEBRATE NESTS	Large vertebrate nest, ø > 80 cm	NE11
		Small vertebrate nest, ø > 10 cm	NE12
	INVERTEBRATE NESTS	Invertebrate nest	NE21
OTHERS (OT) 	SAP AND RESIN RUNS	Sap flow, > 50 cm	OT11
		Resin flow and pockets, > 50 cm	OT12
	MICROSOIL	Crown microsoil	OT21
		Bark microsoil	OT22

Supplementary Material 2

For this work, multispectral information extracted from the Sentinel-2A satellite developed by the European Space Agency (ESA) and thermal data from the Landsat 8 OLI satellite developed by the North American Space Agency (NASA) was collected through https://earthexplorer .usgs.gov/.

The Sentinel-2A satellite allows the highest free spatial resolution available (10 m), offering great potential for vegetation analysis. The level of correction for Sentinel-2A satellite data made available to users is Level 1C (Top-Of-Atmosphere - TOA), which is radiometrically and geometrically corrected products in the UTM / WGS84 projection. Thus, it was only necessary to correct the atmospheric effects, moving to a Level 2A (Bottom-Of-Atmosphere - BOA) product. The atmospheric correction procedure was performed with the Sen2Cor plugin (Sen2Cor, v2.1.2), a Sentinel-2 toolbox (SNAP, 5.0.7), which allows you to process Level 1C data for a Level 2A product. Then, the Normalized Difference Vegetation Index (NDVI) was calculated, which has been shown to be a good measure to quantify the vigor of the vegetation, and is calculated by the following equation:

$$\mathrm{NDVI} = \frac{\mathrm{NIR} - \mathrm{Red}}{\mathrm{NIR} + \mathrm{Red}}$$

where NIR corresponds to the near infrared band (average wavelength 842 nm); and Red corresponds to the band of red (mean wavelength 665 nm). The values of this index range from -1 to +1, and negative values correspond to water, values between 0.0 and 0.49 correspond to impermeable surfaces interspersed with some areas with vegetation, while values greater than 0.5 represent vegetation. dense with high vigor (Ha and Weng 2018).

For this index not to be representative of a single day, a composite of five calculated NDVIs was made, that is, the average of the five NDVIs was calculated and a single spectral image representing the summer NDVI and another representative of the one was obtained for winter (Table 1).

Table 1 – Information regarding the data utilized for the NDVI analysis

Sensor	Resolution	Date	Cloud Coverage	Coordinate System
Sentinel 2A	10 m	14/07/2017 at 11:21:14	1.1364 %	WGS 1984 – UTM 29N
		24/07/2017 at 11:24:33	0.0141 %	
		13/08/2017 at 11:24:33	0.0000 %	
		02/09/2017 at 11:24:31	4.0944 %	
		29/07/2018 at 11:28:45	4.9469 %	
		05/01/2019 at 11:28:19	0.6494 %	
		25/01/2019 at 11:26:24	0.0000 %	
		14/02/2019 at 11:28:01	0.0000 %	
		24/02/2019 at 11:29:23	10.0502 %	
		16/03/2019 at 11:25:01	1.0530 %	

The Land Surface Temperature (LST) plays an important role in many environmental processes as it provides essential information on the physical properties of the earth's surface, allowing the estimation of the urban surface heat island. It should be noted that LST is a proxy for the assessment of the urban heat island, but it boils down to a surface phenomenon that is not equal to its atmospheric counterpart, whose magnitude is smaller during the day and greater at night, the inverse of the LST pattern.

Landsat 8 OLI satellite images were obtained during the most extreme periods of the year (summer and winter) in order to collect contrasting thermal data (Table 2). These images have the highest free spatial resolution available for thermal data (30 m), thus allowing to estimate the surface temperature with high precision. It should be noted that six summer and three winter images were collected, so that the LST was not representative of a single day and thus increased the accuracy of the LST estimate. ArcGIS™ (v.10.5.1) was used to pre-process the raw satellite images, including radiometric calibration, but also the entire process until reaching the final LST equation.

In this work the Radiative Transfer Equation (RTE) model was adopted to estimate LST (Sobrino et al. 2004; Yu et al. 2014). This model essentially requires three parameters: spectral radiance measured by the sensor, terrestrial emissivity and atmospheric transmissivity.

Table 2 – Information regarding the data utilized for the LST analysis

Sensor	Resolution	Date	Cloud Coverage	Coordinate System
Landsat 8 OLI	30 m	02/08/2017 at 11:13:41	4.91 %	WGS 1984 – UTM 29N
		03/09/2017 at 11:13:49	7.68 %	
		18/06/2018 at 11:12:38	0.06 %	
		05/08/2018 at 11:13:03	0.26 %	
		21/08/2018 at 11:13:12	0.02 %	
		22/09/2018 at 11:13:22	0.18 %	
		22/01/2017 at 11:13:50	6.14 %	
		12/01/2019 at 11:13:34	1.25 %	
		13/02/2019 at 11:13:28	0.55 %	

Apparent radiance, that is, surface flux that includes the influence of the atmosphere and surrounding objects that is detected by thermal sensors, is usually converted into digital numbers, which are made available upon acquisition of Landsat images. These numbers were then converted to spectral radiance at the top of the atmosphere and planetary reflectance factors at the top of the solar-corrected atmosphere (for more details see: https://landsat.usgs.gov/using-usgs-landsat- 8-product).

As each type of surface / material has its own emissivity, it was necessary to correct the terrestrial emissivity, estimated from information collected by vegetation indices, such as the NDVI Thresholds Method (Sobrino et al. 2008). This method allows the fraction of materials related to the atmosphere to be determined by evapotranspiration, absorption of solar radiation, energy, etc., thus distinguishing the emissivity between water pixels, soil, mixed materials and vegetation. In addition, since apparent radiance also includes atmospheric effects such as absorption and other emissions along the surface-sensor path, it was necessary to acquire atmospheric parameters to correct these effects, considering the day and time of image acquisition (Barsi et al. 2003; Barsi et al. 2005). These parameters were simulated in the MODTRAN4.0 model. The final LST equation, according to the RTE model, is given by:

$$\mathrm{LST} = \frac{c_2}{\lambda \ln \left\{ \dfrac{c_1}{\lambda^5 \left[\dfrac{L_{TIR} - Lu - \tau\,(1-\varepsilon) Ld}{\tau\,\varepsilon} \right]} + 1 \right\}}$$

where LST is the earth's surface temperature; c_1 and c_2 are constants; λ is the wavelength of the TIR band; L_{TIR} corresponds to the spectral radiance value at the top of the atmosphere of the TIR band; Lu and Ld are the rising and falling atmospheric radiance, respectively; τ is the transmittance and ε the emissivity of the earth's surface.

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SECTION III | CONCLUSIONS AND FUTURE PERSPECTIVES

The results of this dissertation are expected to help identify better conservation measures towards a more sustainable tree management at local and national scales to improve saproxylic biodiversity. This is a first step towards an ecological valorisation of large trees in Portugal achieved within the Green Giants project. Furthermore, complementary, with the work of the VACALOURA.pt project and the Lousada's municipality, deadwood importance is starting to be acknowledge in the country.

This dissertation main results show that a combination of tree traits, applied management and macro/microclimate lead to different TreMs compositions and diversity, influencing therefore, saproxylic biodiversity. Regarding tree traits, native species with large PBH (specially *Q. robur*) have the highest TreMs diversity, fruit of the diversified TreMs assemblages that these trees can host. Contrarily, tall exotic species (e.g., *E. globulus*) have homogenic compositions of TreMs, leading to the lowest values of TreMs diversity in our study area. Tree management also influences TreMs diversity, leading to variations in TreMs compositions according to the land-use where the tree is located. In this case, privately owned grouped/isolated trees that are periodically pruned have the highest TreMs diversity. Forested areas have trees with highly diversified naturalised TreMs compositions, such as deadwood, nests and epiphytes while urban trees have mostly injuries and cavities, resulting of high pruning intensity. Since land use artificialization influences local climate, temperature and humidity also influence local compositions of TreMs, leading to consequent variations in its diversity. Further, simplified models based on the tree species, PBH and total height are able to be used to calculate the TreMs diversity at broader spatial scales, helping to acknowledge the potential of TreMs as biological indicators depending on the habitat where the tree is growing (since the compositions of TreMs vary significantly with it).

Our findings are very important since we can identify tree traits that are linked to higher ecological importance. By promoting the preservation of trees that have these characteristics, we are also able to improve our efforts in terms of biodiversity conservation. Further, these results allow to identify and measure the impact of human activity in the ecological role of these trees. With this dissertation, it will be possible to develop evidence-based measures to promote the preservation of this natural patrimony at a local scale but also to identify national general guidelines.

Lousada is a Portuguese municipality that is leading as an example in terms of preservation of natural patrimony and its use to raise awareness regarding nature conservation within its citizens. Regarding large trees preservation, the Green Giants project is not only aiming to identify and characterize all the large trees but also to use their social and ecological value as a mean to promote better conservation practices. At a local scale, Lousada's large native trees should be preserved at all cost, specially *Q. robur*. To achieve it, an integrative strategy should be adopted, allowing the social, economic and consequently ecological valorisation of these trees. This strategy should, above all, create mechanisms to impede the cut of these trees on a long term and promote the grow of smaller trees to reach larger dimensions and consequently develop heterogenic TreMs compositions. The creation of public laws and regulations that are followed by the creation of an economic compensation system with counterpart protection measures for private owners could be a starting point to have fast protection results at this scale. To achieve long term goals, a social valorisation of these trees is necessary and so mechanisms that lead to an increased awareness of the importance of these trees by Lousada's citizens, such as environmental education activities, should be created. To achieve long term goals, a social valorisation, leading to increased awareness of these trees by the citizens, is necessary according to the different land use types and its influence on the tree species distributions and economical mechanisms to promote better conservation practices by private owners might also be one of the strategies, although both counterparts need to be aligned and work together to achieve greater goals, especially in what comes to land use change.

Lousada's large trees can be found mostly in forests and agricultural areas. In forest areas tree species are dominated by *E. globulus* (61% of the municipal forested area), *P. pinaster*, *P. nigra*, with distributed remnants patches of *Q.robur*, cork oaks (*Q. suber*), American oaks (*Q. rubra*) and invasive species such has *Acacia spp.* or *Robinia pseudoacacia.* The great diversity of trees in this land-use leads to diversified saproxylic communities than in other areas, as a result of a higher abundance of TreMs per area (since more trees are available) but also because, as explained before, the variability of taxa leads to variability of habitats. Regarding agricultural areas, large trees are mostly used by citizens for collecting fruits such has walnuts (*Juglans regia*) and chestnuts (*C. sativa*), but also to support wine yards in traditional agricultural methods called "Vinha do Enforcado" using mostly *Platanus sp*., European hakeberries (*Celtis australis)* and *Q. robur*. "Vinha do Enforcado" trees are actively pruned to control the tree growth leading to the formation of a great abundance and diversity of TreMs in these trees, even if the species is exotic and not reaching high PBH. An assessment of the TreMs diversity of these managed trees should be elaborated since they link socio-economical management with ecological valorisation of trees and perhaps an ongoing valorisation of native trees that are able to fulfil this demanded social goals can be used as a integrative measure to improve saproxylic biodiversity of these areas.

In urban centres the most common species are *Platanus sp*. and are managed in a homogeneous way to fulfil social goals. An increased richness of tree species that are able to reach high PBH should be one of the first goals of the municipality, and to achieve fast results, the large urban trees that are not *Platanus sp.* should be preserved at all cost. Furthermore, promoting different management mechanism can lead to an ecological valorisation of the current large tree, where even creating, in some cases, artificial habitats can help (Le Roux et al. 2014). Residential areas and old farms with big gardens, that are part of the rural landscape, are mostly dominated by large old trees such has *Q. robur, Tilia sp*., *Cupressus sp*. and other botanic rarities that lead to a variation of TreMs compositions that is integrated in the surrounding agricultural or forested environment. These farms allowed the preservation of trees during long periods of time and its possible to find in them some particular specimens of outstanding dimensions and TreMs diversity. The ongoing work with private landowners, with small or large areas, should continue and be improved since it has a high potential of TreMs preservation on a long term.

At a national scale, a broader perspective should be adopted and an improvement of the already ongoing work to identify and preserve large trees is necessary. For instance, in the United Kingdom more than 160 000 large trees were identified by citizens (https://ati.woodlandtrust.org.uk/) and guidelines for the management of these trees were created based on that information. In this sense, Portugal needs to change the way it manages forests and particularly large tree, promoting better preservation and conservation measures. A major problem regarding large trees preservation is that it should not only be based regarding the socio-economical value of trees (what is done nowadays in Portugal) but also regarding their ecological role. The 470 single and 81 groups of monumental trees identified are lacking a management plan, even if mostly are publicly owned (Lopes et al. 2019). There is also lacking a TreMs level characterization and an assessment of their ecological importance. The inventory of TreMs is recommended in forest inventories as a complementary biodiversity indicator since they have been identified as a more direct predictor of forest biodiversity than large structural elements in forest systems, such has deadwood volume (normally used nowadays in Europe) and actually link management abandonment, abundance of large structural elements and biodiversity for several groups and taxa (Paillet et al. 2018). The large scale TreMs assessment might help to valorise these trees, showing that they are vital for nature conservation at large scales.

Although some trees are protected by law at a national scale, thousands of individuals are cut yearly because of land use changes and if they naturally die, they can then be removed. The most important tree species in terms of ecological value in our study was *Q. robur,* a species that does not have any national protection, although some municipalities (10 out of 278 in Portugal) created local laws to prevent the cut of the last few large specimens. Regarding tree preservation at local scale, we promote the preservation of native large trees, especially *Q. robur* in the Temperate/Atlantic biogeographical region of Portugal (Fig. 2.1) where is mostly found, as a ground base to promote an increase in biodiversity conservation in this region. Further, we encourage municipalities to improve the tree species diversity of their urban areas and complementarily improve and vary their management to promote a most heterogenic and rich TreMs composition in these areas, promoting urban biodiversity and increasing the benefits that humans can have from it.

Overall, to change future perspectives regarding tree preservation in Portugal we propose:

1) A change of concept to identify and protect trees, allowing the valorisation and conservation of large trees in Portugal based also on their ecological value;
2) A TreMs assessment of the already identified monumental trees allowing to understand the relation between their already valued social importance and their ecological role;
3) A continued TreMs assessment of large trees of more species and habitats, helping to improve the predicting models;
4) The creation of a national scale large tree identification and characterization database possibly also feed by citizen-science with continuous monitoring;
5) The promotion of social valorisation of these trees using them as touristic attractions or as live laboratories to educate regarding nature conservation and natural heritage;
6) The creation of economic valorisation to these trees as a mean to protect them from being cut without valid reasons.

My future perspectives are linked to both local and national objectives. Regarding local, during the next year I expect to take the next steps towards the preservation of the identified large trees by developing management plans, regulations and mechanisms to improve their preservation. I hope that, in a not so distant future, I can also start replicating the Lousada's efforts in terms of deadwood and large trees preservation at a regional and national scale and by collaborating with other national entities to advance national policy of large trees conservation. Eventually I aim to start a PhD project with the purpose of linking these topics with people awareness towards biodiversity long-term preservation and valorisation.

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